PHYSICAL CHARACTERIZATION METHODS

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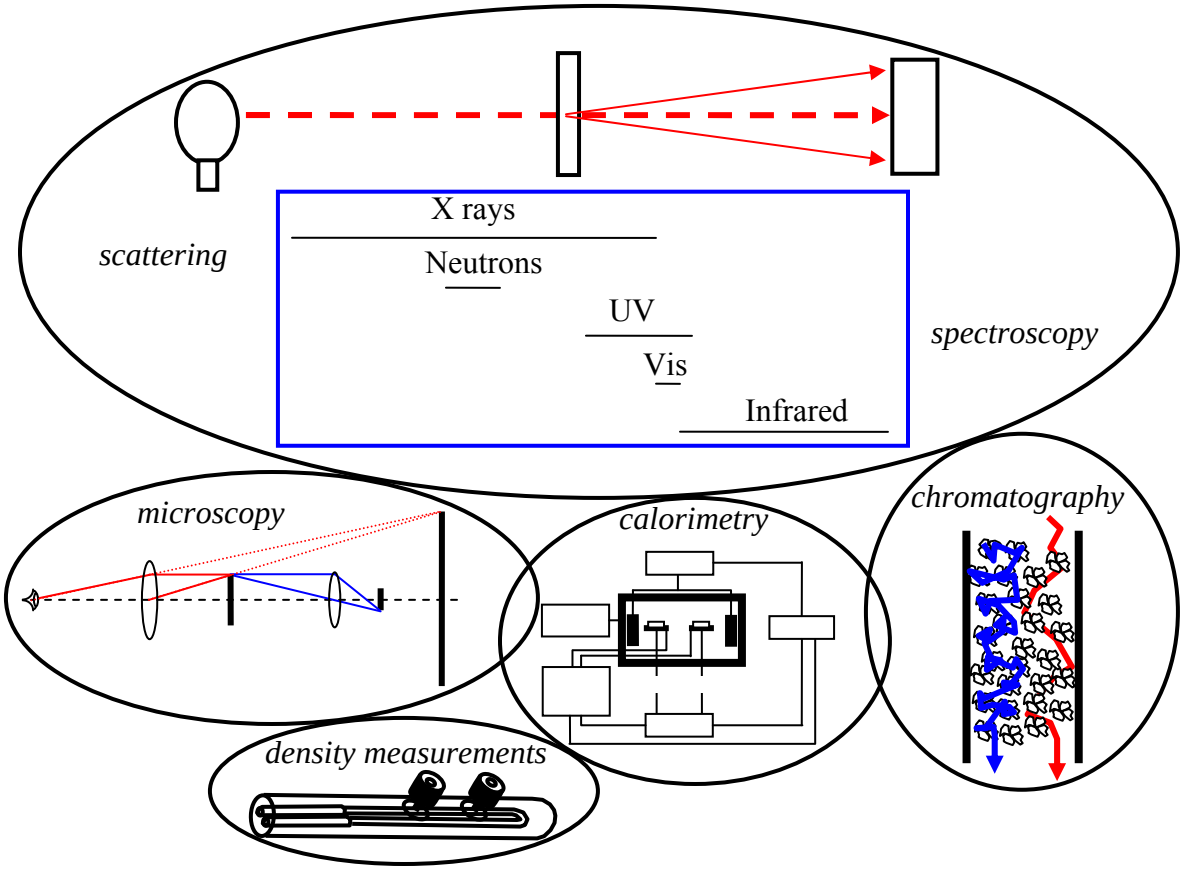
http://www.ncnr.nist.gov/staff/hammouda/physical_characterization_methods.pdf



OUTLINE

INTRODUCTION TO THE CHARACTERIZATION METHODS

A host of characterization methods are used to investigate nanoscale structures at the morphological and/or molecular levels. These are listed here into broad categories: scattering methods (SANS, SAXS, WAXS, DLS, etc), spectroscopic methods (UV-Vis, FTIR, Raman, etc), microscopy (optical, TEM, SEM, etc), calorimetry (DSC, etc), chromatography (GPC, SEC, etc), density measurements, rheology, etc. Some of these methods are briefly described here.

SCATTERING METHODS

This author's research interests are in Small-Angle Neutron Scattering (SANS). This characterization method probes from the near atomic (nanometer) to the near optical (micrometer) length scales and is based at a few neutron scattering research labs. It has had major impact on research in the following fields of research: polymers, complex fluids, biology and materials science.

Small-Angle X-ray Scattering (SAXS) probes a size scale comparable to that of SANS. X-rays, however, are scattered from the electron cloud around atoms and are therefore characterized by different contrast factors than neutrons. SAXS and SANS complement each other with SAXS characterized by high fluxes and easy access to beamtime and SANS characterized by the advantage of partial deuteration.

Dynamic Light Scattering (DLS) is the technique of choice for investigations of diffusive modes in soft materials. It is also used to measure "particle" sizes and size distributions in solutions.

Wide-Angle X-ray Scattering (WAXS) monitors the molecular structure at the local "chemical" level. It is effective in detecting and quantifying the amount of crystallinity in semi-crystalline materials. The scattering variable range is higher than small-angle scattering methods.

Scattering methods are based in the Fourier transform (so-called reciprocal) space. This is different from microscopy methods which are based in the direct space. The reciprocal space is hindered by the phase problem. Taking the inverse Fourier transform of 2D scattering data does not produce a unique picture in direct space whereas the Fourier transform of a microscopy picture produces a unique data set in reciprocal space.

VARIOUS RADIATIONS USED FOR SCATTERING

Many forms of radiation can be used for scattering purposes: X-rays, neutrons, electrons, laser light, gamma rays, etc. These have different characteristics and are used for different purposes. A Table summarizes various scattering methods.

Table 1: Various Radiations Used for Scattering

Type of Radiation	X-Rays	Neutrons	Electrons	Laser Light
Wavelength Range	0.1-5 Å : X-Rays 5 Å -1 μm : VUV	1 Å -15 Å	0.1 Å	1 μm
Sensitive to Inhomogeneities in	Electron Density	Density of Nuclei	Electron Cloud	Polarizability (Refractive Index)
Scattering Methods	SAXS, WAXS	SANS, WANS	LEED	SLS, DLS
Sample Thickness	< 1 mm	1-2 mm	100 μm	1-5 mm
Problems with	Absorption	Low Fluxes	Low Penetration	Scattering from Dust

The small-angle neutron and X-ray scattering methods (SANS, SAXS) probe size scales from the near atomic to the near micron. Light scattering methods (SLS, DLS) complement these techniques by focusing on the micron length scale. Other methods such as wide-angle neutron and X-ray scattering (WANS, WAXS) and low-energy electron diffraction (LEED) probe very local (atomic) structures.

SPECTROSCOPIC METHODS

Absorption and transmission of various electromagnetic radiations are used as spectroscopic characterization methods. The most known of these methods are in the UV-Vis (ultraviolet-visible) and IR (infrared) wavelength ranges. A figure summarizes the spectrum of electromagnetic radiation. X-rays and neutrons are used for scattering methods and have been added for comparison.

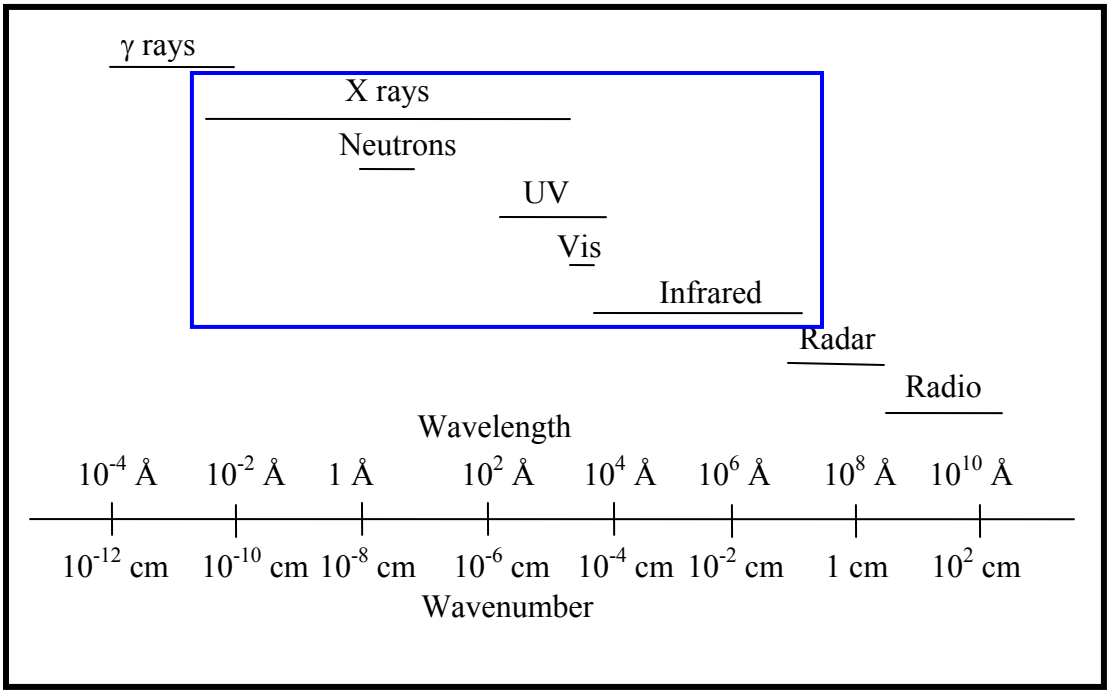


Figure 1: Spectrum of various electromagnetic radiations.

Electromagnetic radiations are characterized by the following relations.

Wavelength denoted λ
Frequency denoted $\nu = c/\lambda$
Wavenumber denoted $k = 1/\lambda$
Energy denoted $E = h\nu$
Planck's constant $h = 6.626*10^{-34}$ J.s $= 4.136 *10^{-15}$ eV.s
Speed of light $c = 3*10^{8}$ m/s

Note that the kinetic energy for particles (such as neutrons) is calculated using a different formula $E = \hbar^2 k^2 / 2m$ where m is the particle mass.

Table 2: Characteristics of the UV-Vis and IR light.

Color	Wavelength in nm	Frequency in 10^{14} Hz	Wavenumber in 10^{4} cm^{-1}	Energy in eV
Infrared	1000	3.00	1.00	1.24
Red	700	4.28	1.43	1.77
Orange	620	4.84	1.61	2.00
Yellow	580	5.17	1.72	2.14
Green	530	5.66	1.89	2.34
Blue	470	6.38	2.13	2.64
Violet	420	7.14	2.38	2.95

Near UV	300	10.00	3.33	4.15
Far UV	200	15.00	5.00	6.20

Absorption spectroscopy instruments require common components for the light source, monochromation, collimation, and detection. Monochromation is often performed using gratings which consist of structures with regular spacings in the submicron to micron size range.

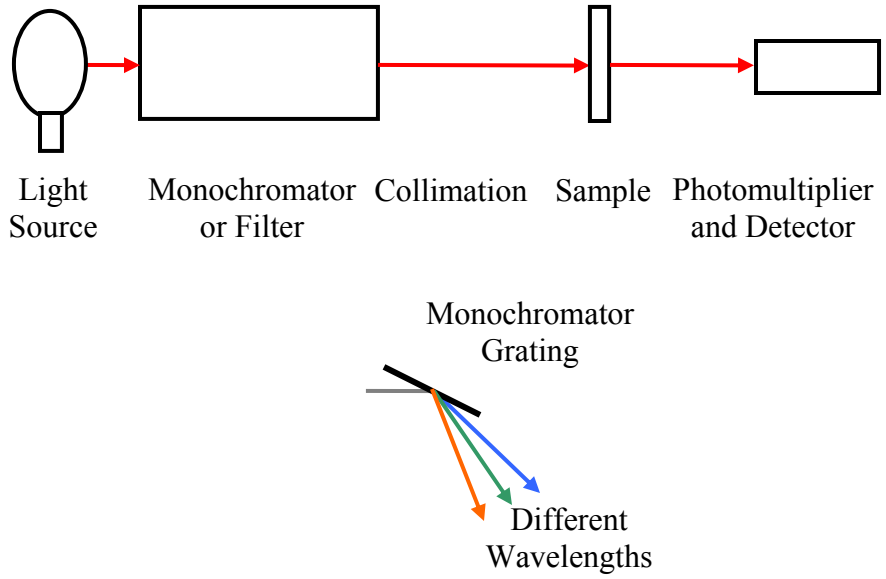


Figure 2: Schematics of a photospectrometer.

MICROSCOPY

Optical microscopy is the conventional form of microscopy. It uses visible light and can observe sizes down to 0.2 micrometer. Modern improvements of this technique include confocal microscopy which consists in focusing on specific layers within the specimen in order to attain depth resolution.

Electron microscopy can observe smaller size scales down to 1 nanometer. Electron microscopy is used in the transmission mode (TEM) for thin samples or in the scanning mode (SEM) to image surfaces. Samples are stained in order to enhance the contrast. Cryo-TEM consists in quenching the sample to low temperature in order to freeze the morphology into thin slices. Cryo-TEM can observe natural contrasts without staining.

CALORIMETRY

Calorimetry consists in measuring the heat emitted and/or absorbed during thermodynamic transitions or chemical reactions. Differential Scanning Calorimetry (DSC) measures the amount of heat absorbed or emitted by two samples undergoing heating and/or cooling cycles: the measured sample and a reference sample. The difference in heat flow constitutes the DSC signal. The measured and reference samples must remain at the same temperature in DSC. In the Differential Thermal Analysis (DTA) method, it is the heat flow to the two samples that remains constant.

CHROMATOGRAPHY

Chromatography is used for fractionation of polydisperse macromolecular systems as well as for measuring molecular weight distributions. It consists in allowing the macromolecules in solution to diffuse down a column with larger sizes diffusing faster. Interaction between the sample and the gel that fills the chromatographic column is the driving factor for diffusion down the column. There are many types of chromatography. Size Exclusion Chromatography (SEC) is very much used.

DENSITY MEASUREMENTS

Density measurements are performed in one of two ways: (1) the old way with a pyrometer or a pychnometer, or (2) the new way using the vibrating U-tube method. The old way consists in direct measurements of the mass of a liquid (relative to that of water) for the same volume. The vibrating U-tube uses an electro-mechanical method to apply oscillations of the tube. As for a vibrating spring, the resonant frequency determines the mass and therefore the density (at fixed volume).

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This tutorial has been put together using multiple sources including a popular online search engine (google) and a much-used knowledge database (wikipedia).

http://www.google.com
http://www.wikipedia.org

SMALL-ANGLE NEUTRON SCATTERING

Small-Angle Neutron Scattering (SANS) probes length scales from the near atomic (nanometer) to the near optical (micrometer) sizes. SANS has been a major characterization method in research areas such as polymers, complex fluids, biology and materials science. It has been ever-growing since its inception some 35 years ago. The ability to use partially deuterated samples is the equivalent of staining in electron microscopy; deuteration has given more specificity to the SANS technique.

THE SANS TECHNIQUE

SANS instruments are about 30 m long and cover scattering angles from 0.1 degrees to 10 degrees in order to resolve a wide size range. SANS requires the use of a neutron source and is therefore based at nuclear research reactors and at spallation reaction facilities. There are three such national labs in the United States. The National Institute of Standards and Technology's Center for Neutron Research is one such facility.

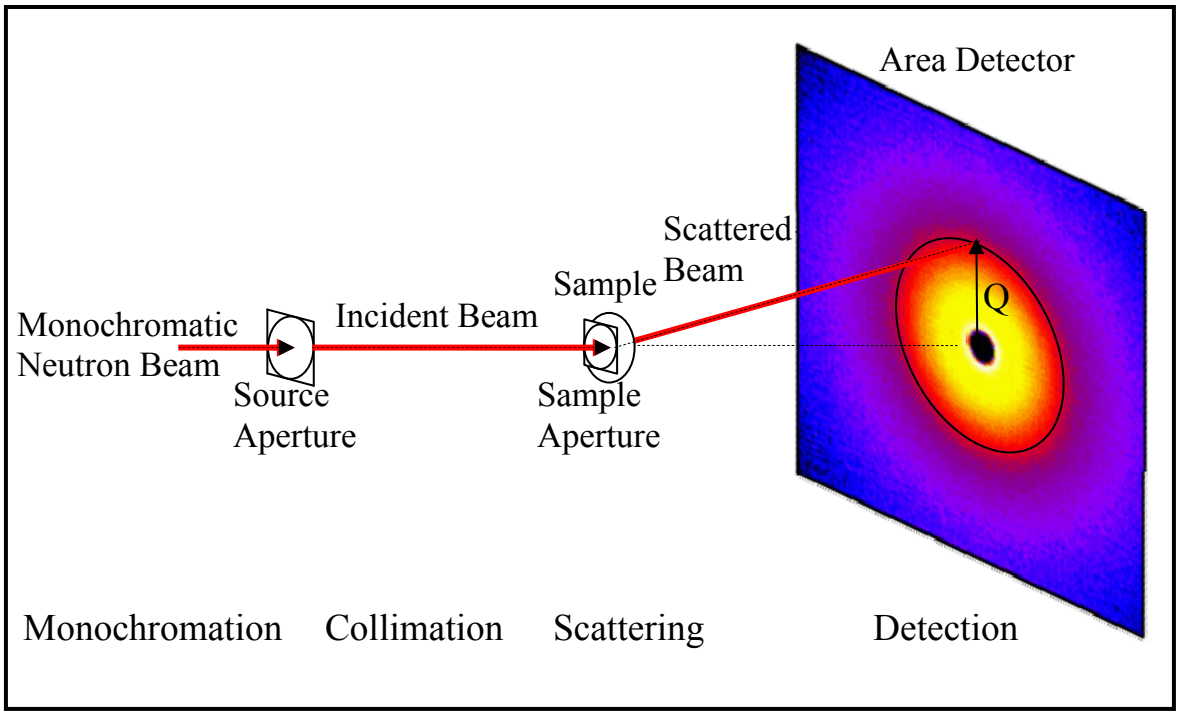

Figure 1: This figure represents the schematics of the SANS technique. It is not to scale; vertical sizes are in centimeters whereas horizontal distances are in meters.

SANS probes size scales from the nearly atomic (1 nm) to the nearly optical (600 nm) size range. This corresponds to a Q-range between 0.6 $Å^{-1}$ and 0.001 $Å^{-1}$. Here Q is the scattering variable expressed in terms of the neutron wavelength λ and scattering angle θ

as $Q = (4\pi/\lambda)\sin(\theta/2)$ .This nanometer size range covers many research areas. SANS data are analyzed using standard plots or fitting to realistic models. For example, the Guinier plot yields the radius of gyration and the Porod plot yields the surface-to-volume ratio of the scattering objects.

SANS RESEARCH TOPICS

Traditionally, the area of polymers has been the most active. This covers polymer blends and solutions, crystalline polymers and the thermodynamics of phase separation in polymeric systems. SANS is an ideal method for probing composition fluctuations and their variation under widely different conditions (of temperature, concentration, and pressure) and has therefore been at the forefront of polymer thermodynamic studies. For example, it was shown how the Gibbs free energy function is obtained from SANS data from a polymer blend mixture using the so-called Random Phase Approximation model. The various types of phase separation either upon cooling or heating were investigated in detail.

The name "complex fluids" is commonly used for fluids that self-assemble due to hydrophobic/hydrophilic interactions. For example, mixtures of surfactant, water and oil form micelles at the nanoscale level. The SANS technique has had major impact on research in complex fluids and other self-assembling systems. The demixing phases of ternary micellar phases were explained. SANS from nonionic as well as ionic micellar systems were investigated. Structural information such as particle sizes and shapes were extracted from SANS data. This was done for spherical, cylindrical and lamellar morphologies. A specific case involves pluronics that form spherical micelles owing to their hydrophobic core and hydrophilic shell. The distribution of water molecules in such micelles has been mapped out precisely. Shearing of such micelles forms cubic single-crystal type structures.

SANS in biology research has been growing over the past ten years. It has focused on membrane work, conformational transitions in DNA and protein complexes. The ability to deuterate portions of such complexes has yielded unique structural information. For example, a kinase protein (KinA) regulates the sporulation process in some bacteria. Sporulation is the ability to package genetic material in response to harsh environment. Spores survive in a dormant state till conditions become more favorable for reproduction. The sporulation process is inhibited through another protein (Sda) that stops the sporulation process. Using deuterated Sda in a KinA-Sda complex, SANS data showed that the inhibition process proceeds through an allosteric action; i.e., it is activated remotely through a four-helix bundle located on the KinA stalk. Another SANS project showed the helix-to-coil conformational change for the DNA denaturation transition. The coil phase was found to contain fully swollen macromolecules.

A collection of other SANS research topics has been covered. These include the effect of pressure on the phase transition of polymer mixtures. Pressure can favor either mixing or demixing in polymer blends and in polymer solutions depending on the enthalpy change

and volume change upon mixing. The Clausius-Clapeyron equation helps in predicting the observed trends. The effect of in-situ shear is to align nanoscale structures as well as shift phase boundary lines. The smectic-to-nematic-to-isotropic lines were seen to shift in lyotropic liquid crystals.

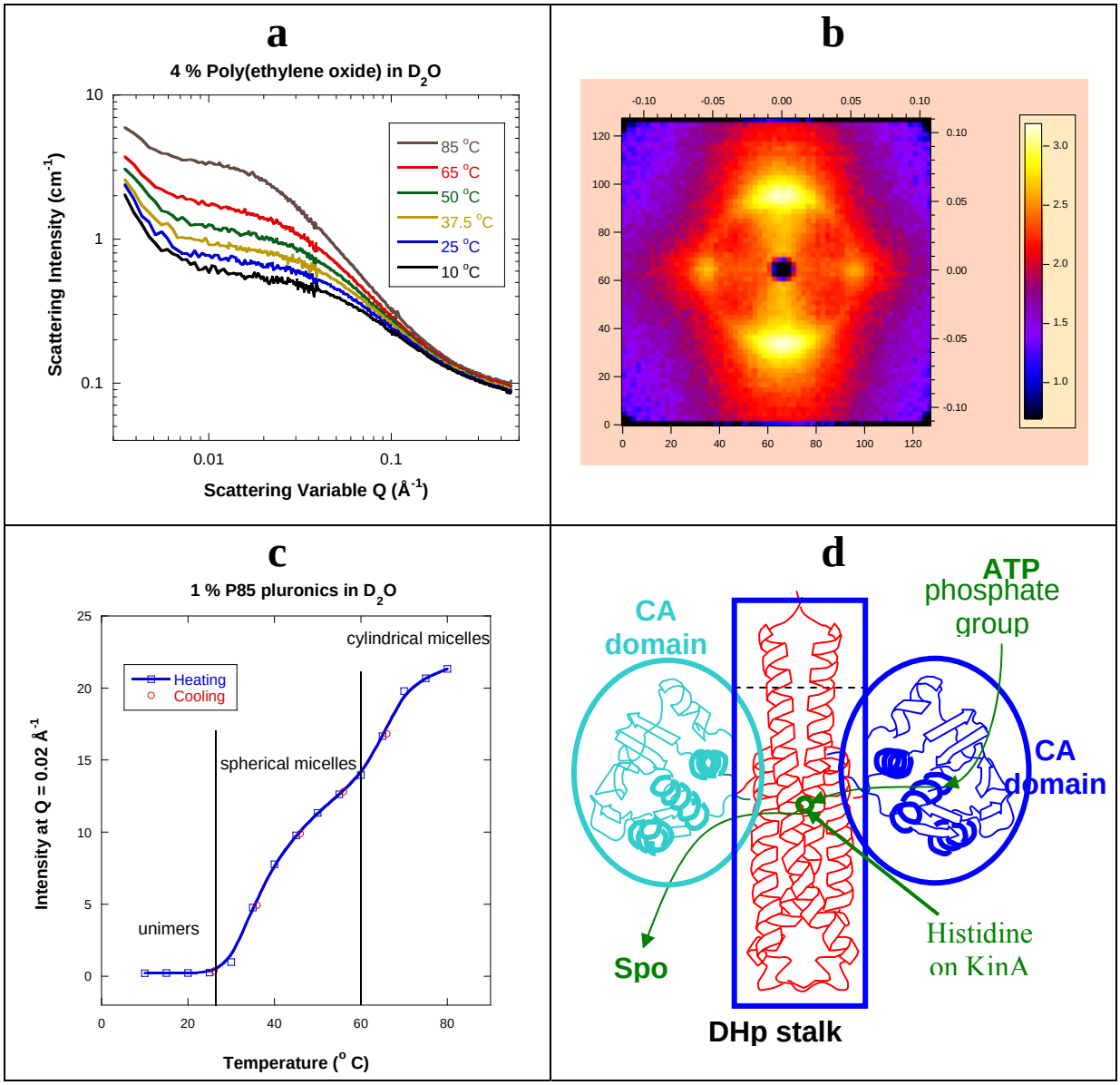

Figure 2: Assortment of representative figures (a) SANS data from a polymer solution (4 % polyethylene oxide in D_2O), (b) SANS image for a sheared complex fluid (the AOT surfactant forms multilayer vesicles in brine/D_2O solvents) (c) low-Q SANS data showing the formation of spherical and cylindrical micelles (pluronics PEO-PPO-PEO triblock copolymers in D_2O) and (d) schematic representation of a sporulation kinase protein called KinA.

Some recent SANS studies from model synthetic and biopolymer solutions are available (Horkay-Hammouda, 2008). Moreover, a 670 page book put together by this author and covering most aspects of SANS is available online (Hammouda, 2008).

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F. Horkay and B. Hammouda "SANS From Model Synthetic and Biopolymer Solutions", Colloid and Polymer Science 286, 611-620 (2008)

B. Hammouda, "Probing Nanaoscale Structures: The SANS toolbox", (2008). This is a 670 page book covering most aspects of SANS. The pdf file is available online at http://www.ncnr.nist.gov/staff/hammouda/the_SANS_toolbox.pdf

QUESTIONS

1. What is the size range probed by SANS?
2. What are the main SANS research areas?
3. Why is the SANS technique a good probe for thermodynamic studies?
4. What is the main advantage of the SANS technique?
5. What can be obtained from a Guinier plot?

ANSWERS

1. SANS probes inhomogeneities in the nanometer size range (from 1 nm to 0.6 nm).
2. The main SANS research areas are: polymers, complex fluids, biology and materials science.
3. The SANS technique is sensitive to density and composition fluctuations. These get enhanced close to phase transition lines making the SANS technique a good thermodynamics probe for mixing/demixing phase diagrams.
4. The main advantage of the SANS technique is the ability to deuterate part of the sample (specific molecules).
5. A Guinier plot yields the radius of gyration.

SMALL-ANGLE X-RAY SCATTERING

The closest characterization technique to Small-Angle Neutron Scattering (SANS) is Small-Angle X-ray Scattering (SAXS).

THE SAXS TECHNIQUE

The SAXS instrument consists of an X-ray source, a pinhole or a slit collimation system, a sample environment, a scattering vessel and a 2D detector. The X-ray source is either a laboratory source like a Kratky or a rotating anode (whereby X-rays are produced when charged particles hit a Cu or a Mo target), or a synchrotron facility (whereby X-rays are produced by accelerated particles moving in a magnetic field). The pinhole collimation is preferred but is characterized by low flux-on-sample. Slit collimation yields higher fluxes but produces slit-smeared data. For example, most laboratory X-ray sources use slit collimation whereas most synchrotron sources use pinhole collimation. The Cu-Kα line is characterized by a source wavelength of 1.54 Å.

SAXS DATA FROM A PROTEIN COMPLEX

SAXS was performed on a protein complex (sporulation kinase called KinA and its inhibitor called Sda). The idea was to investigate the sizes of the two individual components and of the complex.

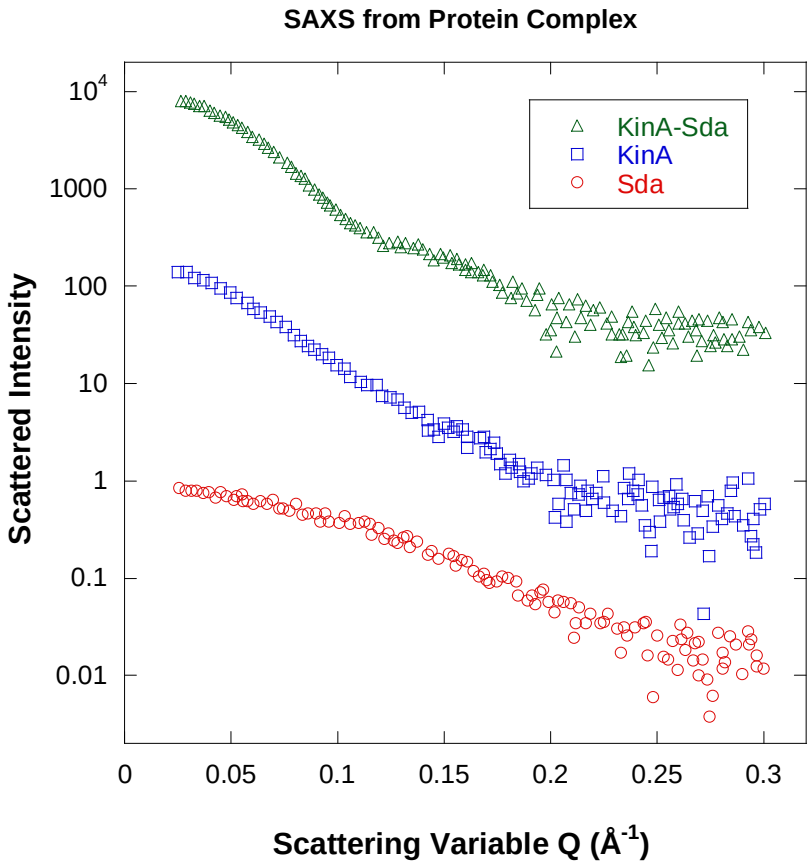

Figure 1: SAXS from dilute solutions of KinA and Sda alone and of the KinA-Sda complex. Curves have been shifted arbitrarily upward to avoid overlap.

The pair-distance probability distribution function $P(\vec{r})$ is the inverse Fourier transform of the scattering form factor P(Q). The distance distribution function $4\pi r^2 P(\vec{r})$ (also referred to as the pair correlation function) was obtained. It represents an estimate of the average size of the KinA, Sda and KinA/Sda complex (peak position) and goes to zero at the particle edge (at D_{max}).

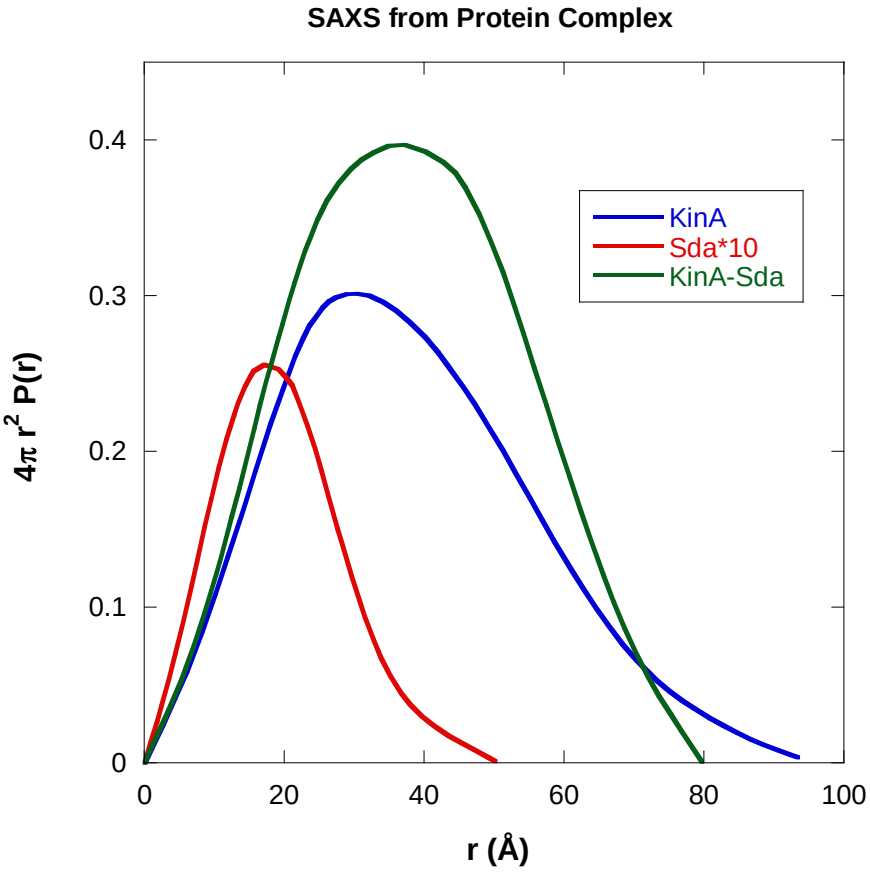

Figure 2: Distance distribution functions obtained from SAXS data from the individual protein and from the protein complex. Scattering from Sda was scaled up (*10).

The radius of gyration is obtained as the second moment of $P(\vec{r})$. R_gs for KinA and Sda were obtained from the Guinier analysis and from the second moment of P(r) analysis and are summarized in a table. Sizes obtained for the KinA/Sda complex showed a compaction of KinA after Sda binding.

Table 1: Size parameters derived from the SAXS Guinier and P(r) data analyses.

Sample	Concentration (mg/ml)	Guinier R_g (Å)	R_g from P(r) (Å)	D_{max} from P(r) (Å)
KinA	3.7	29.3	29.6	95
Sda	5.2	15.3	15.4	52
KinA/Sda	4.4	29.2	29.1	80
KinA/Sda	3.7	29.4	29.1	80

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QUESTIONS

1. X-ray interactions are mostly with what part of the atom?
2. What are the main types of X-ray sources?
3. Old SAXS instruments used slit collimation. What is the disadvantage of slit collimation?
4. What is the pair correlation function?
5. Think of a way of changing sample contrast with SAXS.

ANSWERS

1. X-ray interactions are mostly with the electron cloud.
2. The two main types of X-ray sources are: rotating anode and synchrotron.
3. Slit collimation produces slit-smeared data.
4. The pair correlation function is the Fourier transform of the scattering intensity.
5. A way of changing sample contrast with SAXS would be to use heavy atoms. This, however, is a drastic effect on sample properties.

DYNAMIC LIGHT SCATTERING

INTRODUCTION TO DYNAM IC LIGHT SCATTERING

Dynamic Light Scattering (DLS) is a good monitor of diffusive processes in soft matter (for example macromolecular solutions). This technique, also called quasielastic light scattering or photon correlation spectroscopy (Schmitz, 1990; Chu, 1991; Berne-Pecora, 2000) measures the correlation in time of a laser beam scattered from the sample at a fixed angle. Macromolecules undergo a diffusive Brownian motion when in solution. This is manifested as a time-dependent fluctuation of the scattering intensity. The intensity autocorrelation function is given as:

$$g^{(2)}(Q,t) = \frac{< I(Q)I(Q,t) >}{< I(Q)I(Q) >}. \qquad (1)$$

This second order (intensity) correlation function $g^{(2)}(Q,t)$ is expressed in terms of the first order (electric field) correlation function $g^{(1)}(Q,t)$ as:

$$g^{(2)}(Q,t) = 1 + \beta\left[g^{(1)}(Q,t)\right]^2. \qquad (2)$$

Here β is a factor that depends on the DLS setup. Time correlations are close to unity at short times and decay out at long times. To simplest approximation (and assuming a compact object with diffusion coefficient D_m) correlations die out exponentially:

$$g^{(1)}(Q,t) = g^{(1)}(Q)\exp\left(-Q^2 D_m t)\right). \qquad (3)$$

This assumes one dominant diffusive mode with decay rate $\Gamma = Q^2 D_m$.

The Stokes-Einstein equation relates the diffusion coefficient D_m to the hydrodynamic radius R_H as:

$$R_H = \frac{k_B T}{6\pi\eta D_m}. \qquad (4)$$

T is the sample temperature in absolute units, k_B is the Bolzmann constant, η is the sample viscosity, and "stick" boundary condition has been assumed. In the general case of multiple diffusive modes there is a sum of exponential decays with fractions $G_i(\Gamma_i)$ and decay rates Γ_i:

$$g^{(1)}(Q,t) = \sum_{i=1}^{n} G_i(\Gamma_i)\exp(-\Gamma_i t) = \int_0^\infty d\Gamma G(\Gamma)\exp(-\Gamma t). \qquad (5)$$

A cumulant analysis method approximates this normal mode expansion as follows:

$$g^{(1)}(Q,t) = \exp\left(-\overline{\Gamma}t\right)\left[1 + \frac{\mu_2}{2!}t^2 - \frac{\mu_3}{3!}t^3 + \ldots\right]. \qquad (6)$$

Here $\overline{\Gamma}$ is the decay rate for the dominant mode, $\mu_2/\overline{\Gamma}^2$ is the second order polydispersity index, etc.

A numerical approach based on an inverse Laplace transform known as the CONTIN analysis method works well for polydisperse multimodal systems which cannot be resolved with the simple cumulant method. This approach yields a multimodal distribution of sizes (R_H) from the measured time correlation function.

DLS FROM A POLYMER SOLUTION

DLS measurements were made from a poly(ethylene oxide) solution in water (Ho et al, 2003). PEO with a weight-average molecular weight (M_w) of 100,000 g/mol and a number-average molecular weight (M_n) of 96,000 g/mol was dissolved in freshly double-distilled deionized (DI) water (pH of 6.4) at a concentration of approximately 0.025 wt %. Solutions were allowed to reach equilibrium overnight under ambient conditions before the DLS measurements. A DynaPro DLS instrument with a laser wavelength of 7827 Å and a scattering angle of 90 ^{o} was used.

Sample filtering is an almost required practice for DLS studies in order to avoid overwhelming scattering from "dust" particles. These are formed of undesired material such as initiator, impurities, etc. SANS studies showed that PEO/d-water solutions are characterized by a low-Q clustering mode and a high-Q solvation mode. Q is the scattering variable defined in terms of the wavelength λ and scattering angle θ as $Q = (4\pi/\lambda)\sin(\theta/2)$. We wanted to characterize these two modes by DLS.

The measured time correlation function $g^{(2)}(t)$ is plotted before filtering the PEO/water solution, just after filtering and then a couple of days after filtering.

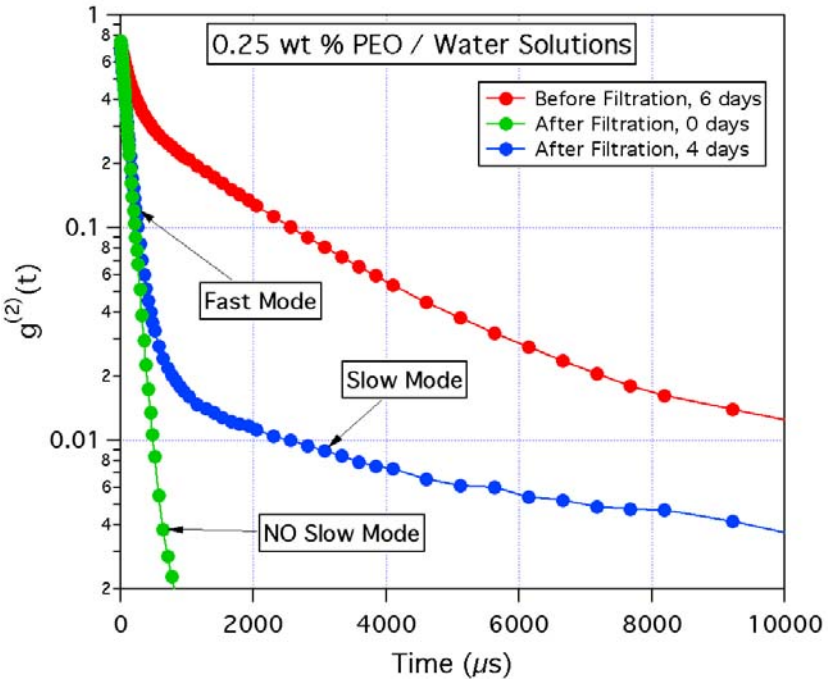

Figure 1: Measured time correlation function.

DLS time correlations show a "fast" mode and a "slow" mode. The CONTIN type data treatment produced a distribution of sizes (R_H) for the three cases considered.

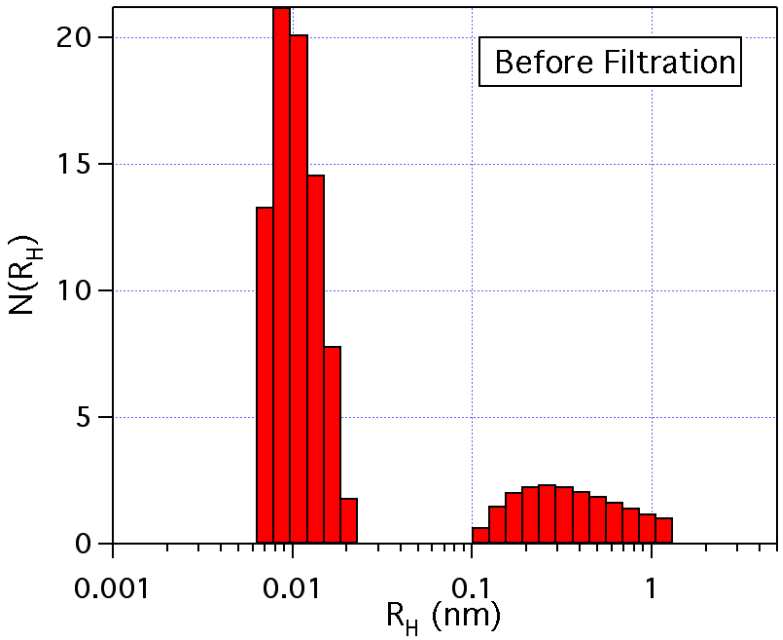

Figure 2: Distribution of R_H before filtering of the PEO/water solution.

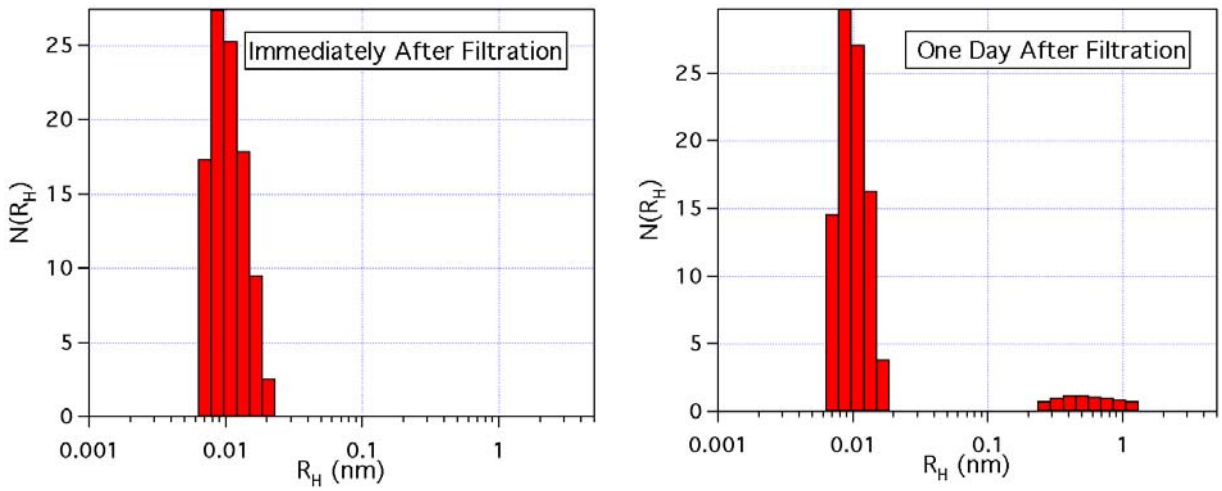

Figure 3: Distribution of R_H after filtering of the PEO/water solution.

The DLS results show that the slow mode disappears with filtering but then reappears with time. The slow mode is due to chain entanglements.

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QUESTIONS

1. What are other names for Dynamic Light Scattering?
2. What can be measured using Dynamic Light Scattering?
3. How is the hydrodynamic radius obtained?
4. What is the essential sample preparation step before DLS measurements?
5. What is the characteristic wavelength of light? What does this imply about light scattering in general?

ANSWERS

1. Dynamic light scattering is also referred to as Quasi-Elastic Light Scattering or Photon Correlation Spectroscopy.
2. Dynamic Light Scattering measures mainly the diffusion coefficient of particles or macromolecules. This can be related to the hydrodynamic radius.
3. The hydrodynamic radius is obtained from the Stokes-Einstein relation relating it to the diffusion coefficient.
4. Before DLS measurements, most samples must be filtered in order to remove “dust” particles. These are impurities that produce unwanted background.
5. The wavelength of light is a fraction of a micron. This implies that light scattering can not probe very small (nm size) objects.

WIDE-ANGLE X-RAY SCATTERING

The Wide-Angle X-ray Scattering (WAXS) technique is a diffraction method that consists in scanning the diffraction angle to cover a wide scattering variable down to small d-spacings which correspond to chemical bonds sizes (Warren, 1990). Amorphous structures yield scattering halos (broad peaks) whereas crystalline structures are characterized by Bragg peaks. WAXS is therefore a good monitor of crystallinity in the sample. There are three forms of wide angle scattering: (1) single crystal diffraction, (2) powder diffraction and (3) wide-angle scattering from amorphous structures. Single crystal X-ray diffraction is crucial for the determination of protein structures for crystallizable proteins.

WAXS FROM A SEMICRYSTALLINE POLYMER SOLUTION

Poly(ethylene oxide) crystallizes in ethanol. In order to assess the level of crystallization, WAXS was performed on PEO/d-ethanol. Deuterated ethanol was used to be consistent with SANS data taken on the same system. Most of the scattering is due to scattering from amorphous regions. Two peaks indicate that although the level of crystallization is low, crystallization is real.

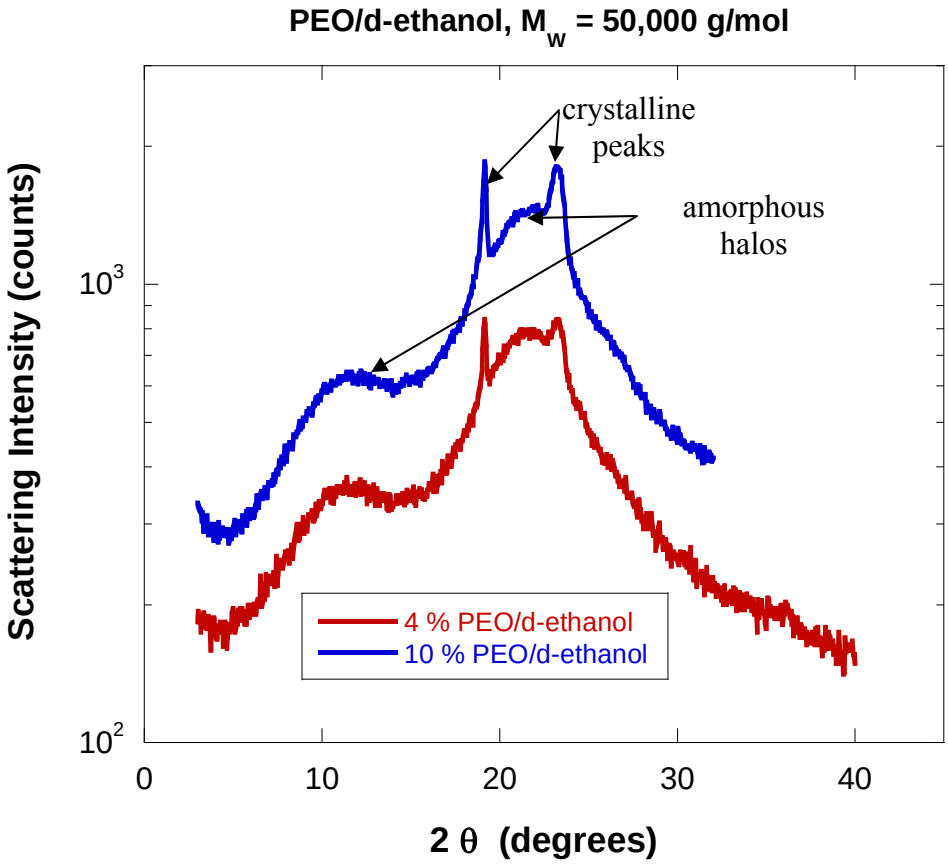

Figure 1: Wide-Angle X-ray Scattering (WAXS) spectra from PEO/ethanol showing crystallinity in the sample. The second spectrum was shifted upward.

To convert to the scattering variable $Q = (4\pi/\lambda)\sin(\theta/2)$ a neutron wavelength of λ = 1.54 Å (Cu-Kα) is used.

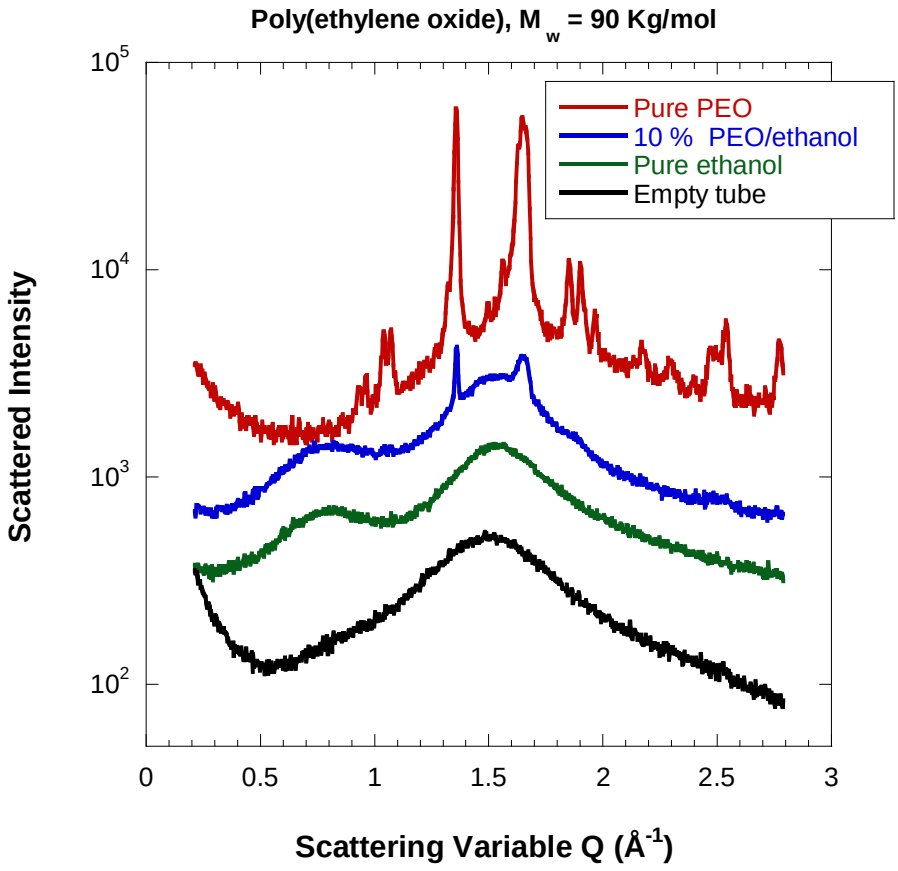

Figure 2: WAXS from the pure PEO, the pure ethanol solvent, a 10 % PEO/ethanol solution and from the empty tube used to contain the sample.

Most of the halo observed at Q = 1.5 $Å^{-1}$ is due to the empty tube and the halo located at Q = 0.75 $Å^{-1}$ is due to the pure ethanol solvent. The crystalline peaks are from poly(ethylene oxide).

Wide angle neutron scattering (WANS) is similar to WAXS but uses neutrons instead (Hammouda et al, 1988).

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QUESTIONS

1. What are the three forms of wide-angle scattering?
2. How large is the scattering angle for wide-angle scattering?
3. What is a Bragg peak?
4. What is the signature of crystalline systems? How about amorphous structures?
5. Name some basic crystalline structures.

ANSWERS

1. The three forms of wide-angle scattering are: (1) single crystal diffraction, (2) powder diffraction and (3) wide-angle scattering from amorphous structures.
2. Wide-angle scattering (or diffraction) involves angles as large as 120 degrees.
3. A Bragg peak is a sharp scattering feature characterizing regularly ordered (periodic) structures.
4. Crystalline structures are characterized by sharp peak whereas amorphous structures show broad halos.
5. The name of some basic crystalline structures follow: body-centered cubic, face-centered cubic, hexagonal, etc.

UV-VIS ABSORPTION SPECTROSCOPY

THE UV-VIS ABSORPTION SPECTROSCOPY TECHNIQUE

The absorption of light in the Ultra-Violet (UV) and visible (Vis) spectrum is an effective spectroscopic method for detecting specific chemical bonds in small molecules and in macromolecules.

The UV-Vis absorption spectrophotometer instrument uses a Xenon pulse lamp which provides a wide wavelength range (from 190 nm to 1100 nm) and a dual silicon diode detector. A holographic grating is used to scan the wavelength.

Absorption depends on three factors: the sample thickness t, the solute concentration c and the absorption coefficient a. The Beer-Lambert expression relates the transmitted intensity to these factors as: $I = I_0 \exp(-a.c.t)$.

The UV-Vis absorption spectroscopy method is used extensively in biology to detect the presence and relative amount of DNA or proteins. For example, this technique is sensitive to the π-bonding in the amine bases of DNA.

THE DNA MOLECULE

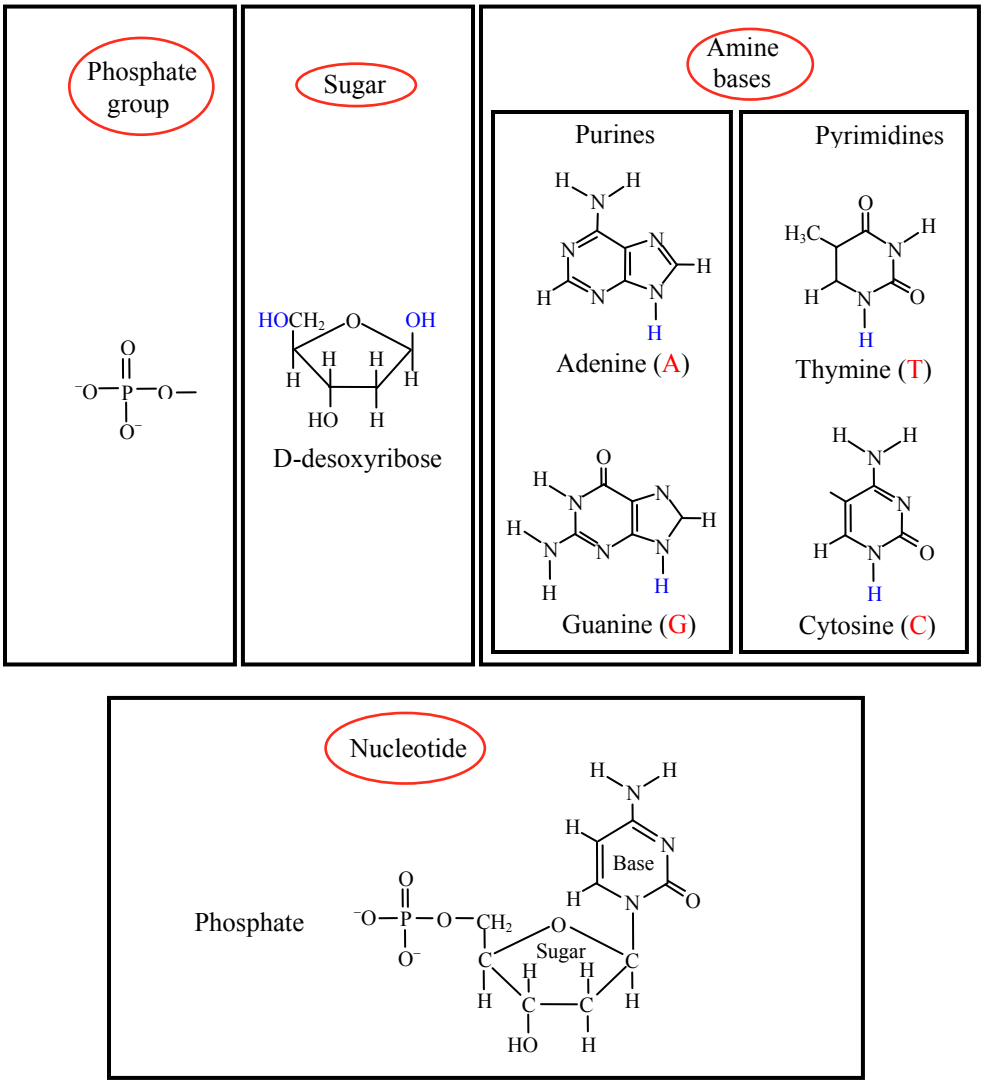


Figure 1: The DNA molecule (Becker et al, 1999).

The π-bonding absorption line occurs at a wavelength around 260 nm for the various nucleotides.

Table 1: Absorption line for the various nucleotides.

RNA	Absorbance Peak Wavelength (nm)	DNA	Absorbance Peak Wavelength (nm)
Adenosine	259	Adenosine	258
Cytidine	271	Cytidine	271
Guanosine	252	Guanosine	---
Uridine	262	Thymidine	267

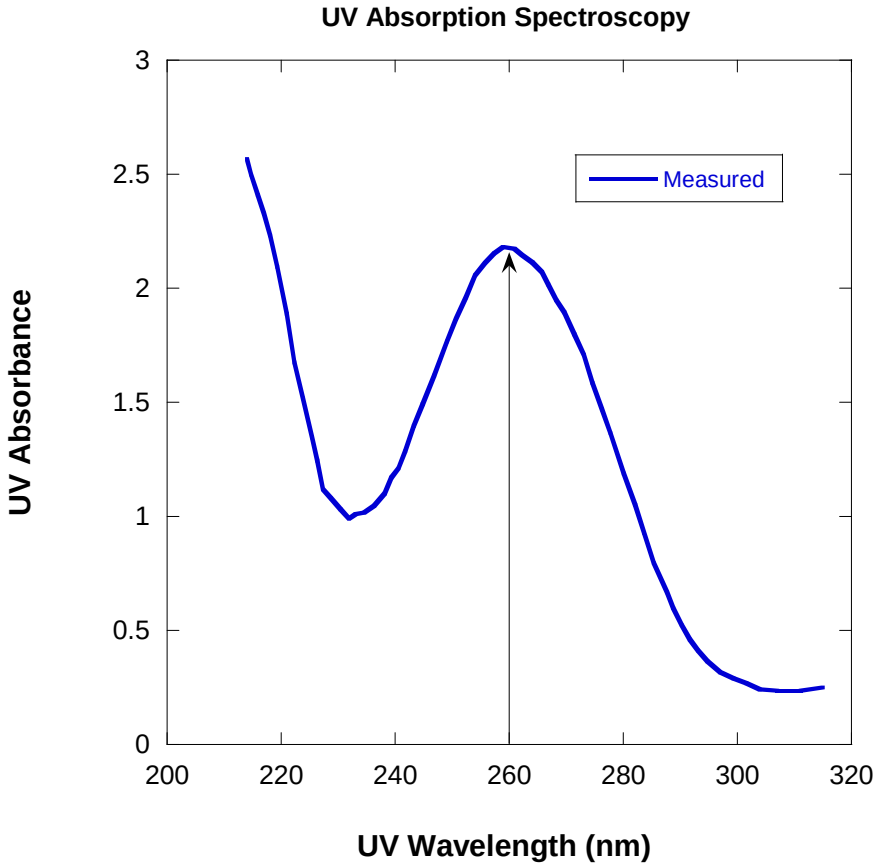
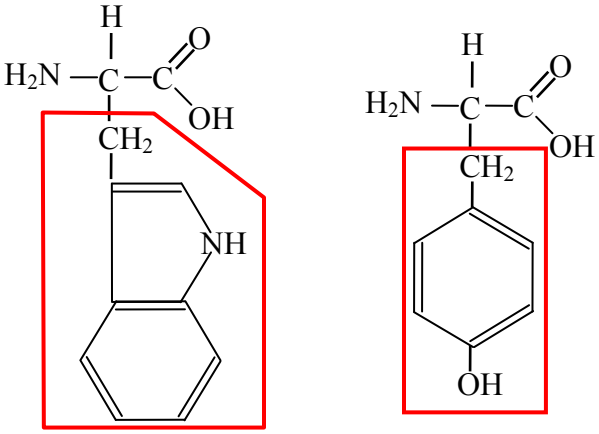


Figure 2: The 260 nm absorption line monitors the stacking of the amine bases in DNA.

The UV-Vis absorption technique is also sensitive to the presence of two amino acids forming the proteins: Tryptophan and Tyrosine.

Note that the absorption signal from proteins (observed around the 280 nm absorption line) is 40 times smaller than that from DNA (observed around the 260 nm absorption line) for comparable concentrations.



Tryptophan
(Trp or W)

Tyrosine
(Tyr or Y)

Figure 3: Chemical formulas for the two amino acids that can be detected by the UV-Vis absorption spectroscopy method.

The absorbance is typically kept between 1 and 10 in order to avoid signal saturation effects. This is done by adjusting the sample thickness and concentration.

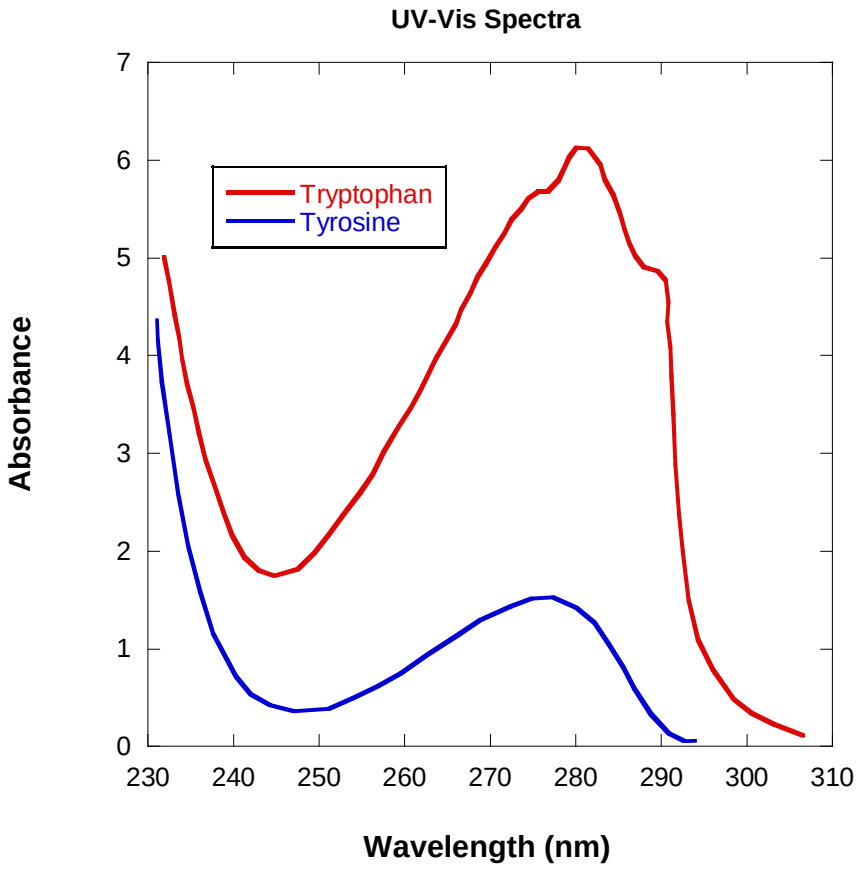


Figure 4: The absorbance of the two UV active amino acids.

The UV-Vis absorption spectroscopy method can also be used to distinguish among the possible macromolecular conformations: alpha helix, beta sheet or random coils. The Circular Dichroism (CD) method is better suited for this task.

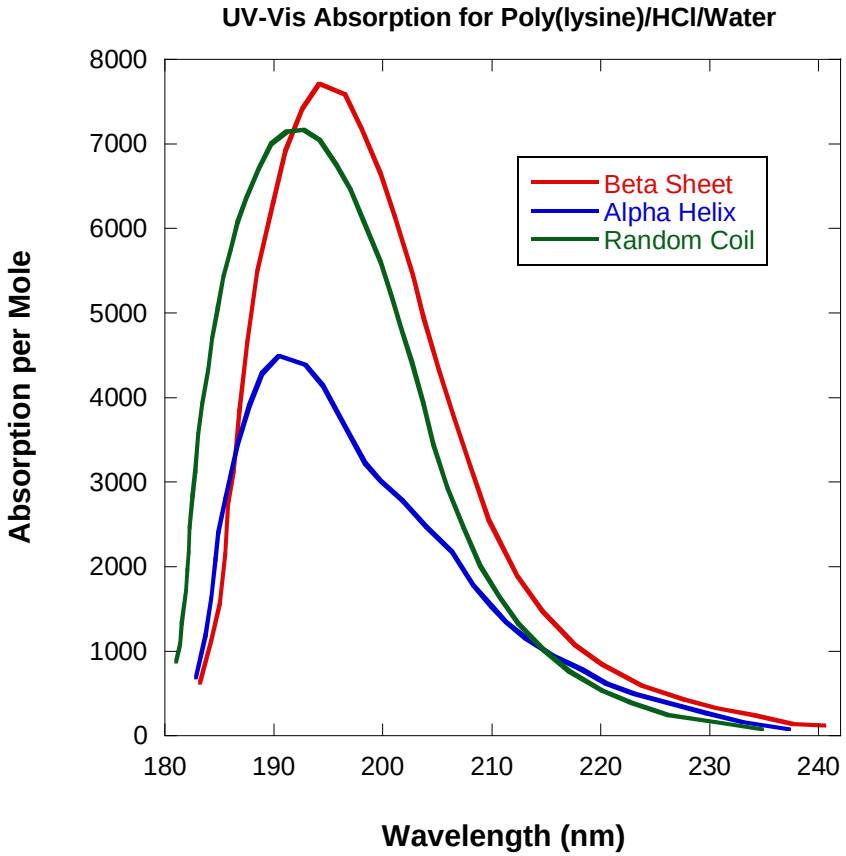


Figure 5: UV spectra for poly(lysine) in HCl/water solution showing the three forms of macromolecular conformations: random coil obtained for pH = 6.0 and T = 25 ^{o}C, helix obtained for pH = 10.8 and T = 25 ^{o}C and beta sheet obtained for pH = 10.8 and T = 52 ^{o}C.

THE HELIX-TO-COIL TRANSITION IN DNA

The conventional method for characterizing the helix-to-coil melting transition in DNA is UV absorption spectroscopy. The 260 nm line is a strong and reliable indicator of amine base stacking (or un-stacking). A Cary 50 instrument was used with a temperature control system. Signal from a 4 % weight fraction sample of salmon DNA was so strong that sample thicknesses around 50 μm were used in order to avoid signal saturation (i.e. to keep the absorbance low).

Signals from 4 % DNA/d-water/0.1M NaCl and 4 % DNA/d-ethylene glycol/0.1M NaCl were measured (Hammouda-Worcester, 2006). Deuterated solvents (d-water and d-ethylene glycol) were used in order to keep consistency with the SANS measurements. A Figure shows the melting curves and transition temperatures in both cases. The transition temperature with the d-ethylene glycol solvent is conveniently located at 38 ^{o}C ± 0.5 ^{o}C, well below the transition temperature with the d-water solvent at 94 ^{o}C ± 0.5 ^{o}C. The melting curves are characterized by a sharp increase of the 260 nm absorption line intensity, then a leveling off. The transition temperature is chosen halfway between these two temperatures (i.e., in the middle of the sigmoid shape).

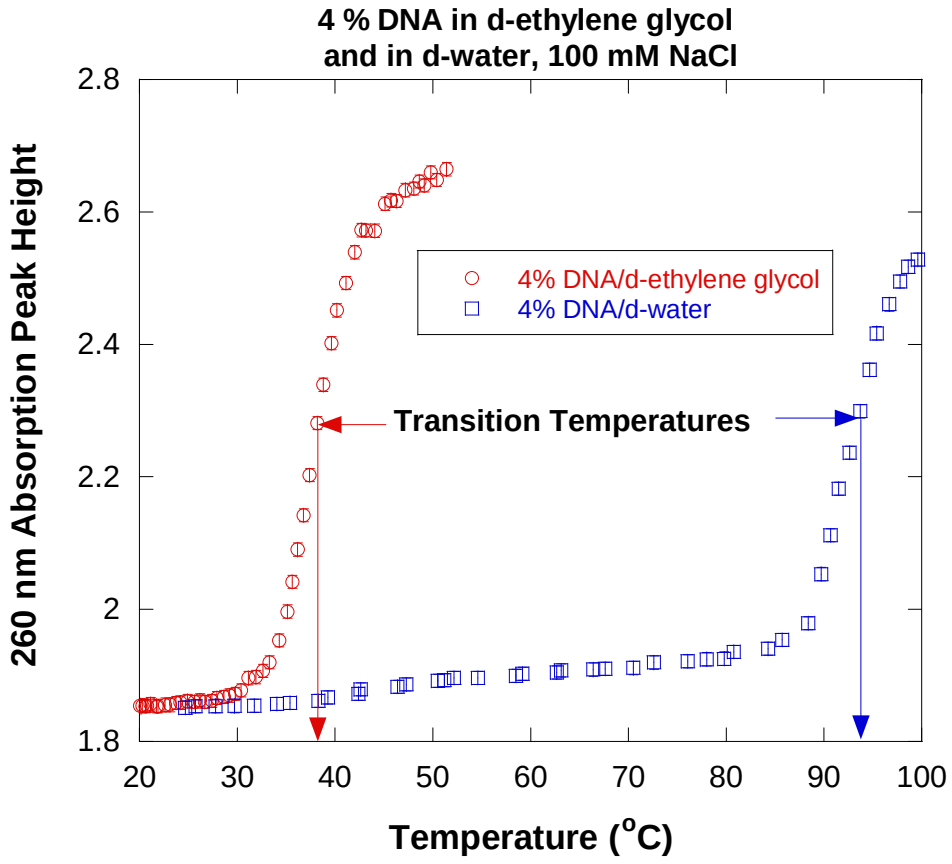


Figure 6: The helix-to-coil transition for 4 % salmon DNA in d-ethylene glycol and in d-water.

A series of UV absorption spectroscopy measurements were made on a series of samples containing various fractions of d-water/d-ethylene glycol. All samples contained 4 % DNA (w/w) and 0.1M NaCl. Figure 7 shows the helix-to-coil melting temperatures in each case along with the two characteristic temperatures (first and second change-of-trend temperatures that give a feel for the transition range). It is seen that the melting temperature follows an almost linear variation (with a slight bend at 80 % d-ethylene glycol fraction) and that the transition range is fairly uniform. The reported transition temperatures were obtained upon heating. This melting transition is reversible although

with strong hysteresis. The monotonic linear variation is attributed to the fact that the transition was approached from the helix side whereby solvents mix randomly (ideal solvent mixing behavior).

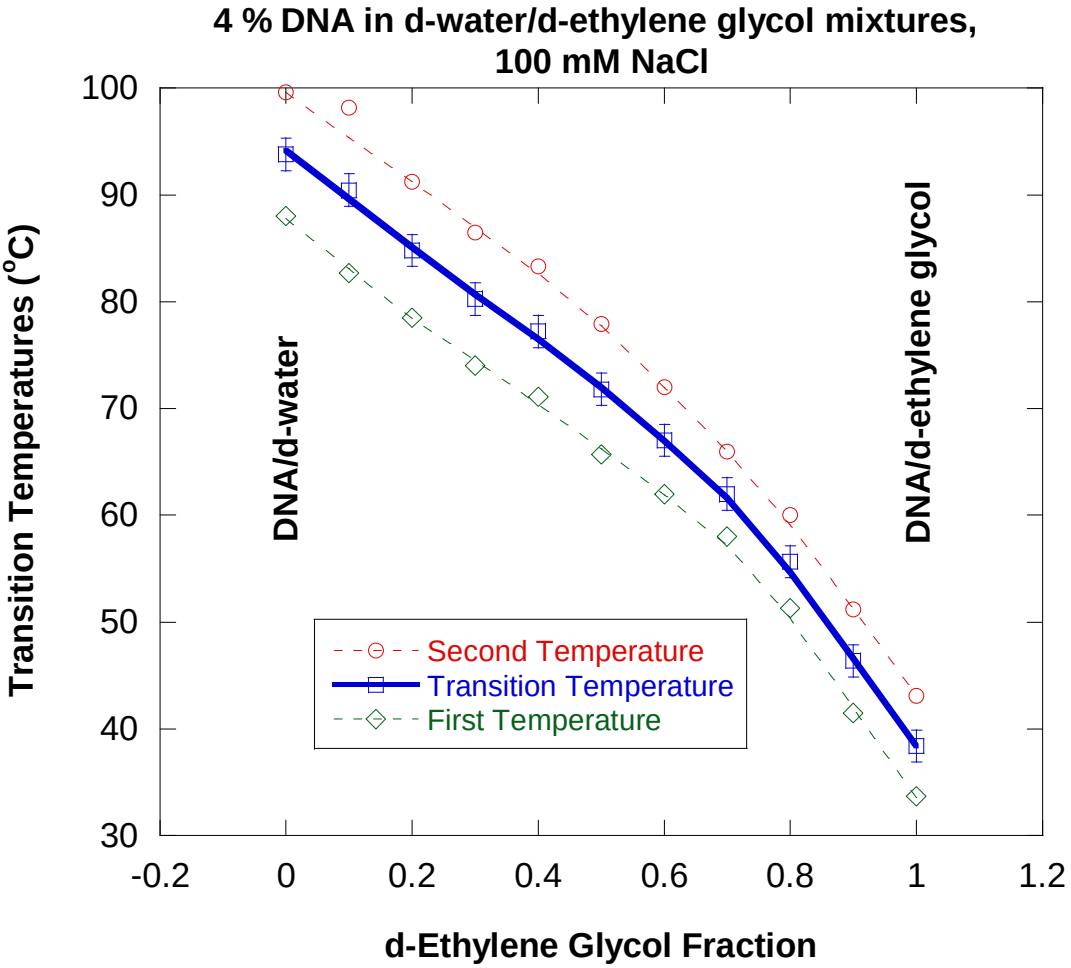


Figure 7: The helix-to-coil transition in mixtures of d-ethylene glycol and d-water.

The fact that the melting temperature shown in the figure decreases with d-ethylene glycol fraction points to the conclusion that the hydrophobic groups CD_2 in d-ethylene glycol play an important role in the melting transition. They help solvent molecules cross the hydrophobic zone of the sugar groups thereby loosening the helical structure (un-stacking of the amine bases and breaking of the hydrogen bonds between these bases).

These observations point to the importance of hydrophilic interactions (around the phosphate groups) and hydrophobic interactions (around the sugar groups) in the melting transition. The solvent's ability to cross the hydrophobic region controls the stability of the helix phase.

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W.M. Becker, L.J., Kleinsmith and J. Hardin, "The World of the Cell", Benjamin/Cummings Publishing (1999)

B. Hammouda and D.L. Worcester, "The Denaturation Transition of DNA in Mixed Solvents", Biophysical Journal 91, 2237-2242 (2006).

QUESTIONS

1. What is the wavelength range of visible light?
2. UV absorption spectroscopy is sensitive to what type of bonding?
3. What is the cause of the 260 nm UV absorption line in DNA?
4. What is the cause of the 280 nm UV absorption line in proteins?
5. How many amino acids are there?

ANSWERS

1. Visible light has wavelengths in the range of 0.4 μm to 0.7 μm.
2. UV absorption spectroscopy is sensitive to π bonding (for example in benzene rings).
3. The 260 nm UV absorption line is due to π bonding from the stacking of the amine bases.
4. The 280 nm UV absorption line is due to π bonding in the Tryptophan amino acid mostly.
5. There are 20 amino acids.

FTIR ABSORPTION SPECTROCOPY

THE FTIR TECHNIQUE

Fourier-Transform Infra-Red (FTIR) spectroscopy is another absorption/transmission method used to probe chemical bonds and their crowding environment in molecular systems. It is a chemical analysis method of choice used to rapidly identify substances; it produces their molecular fingerprint. Absorption peaks correspond to normal mode frequencies of the molecular bonds making up the material. An interferometer is used to encode the detected signal which is digitally Fourier transformed to produce an FTIR spectrum (absorbed intensity *vs* wave number).

The temporal coherence of the light source (IR in this case) is measured using a Michelson interferometer method with two reflecting mirrors; one fixed and one moving. The moving mirror introduces a time delay and therefore allows scanning of the time variable in the absorbance/transmittance intensity I(t).

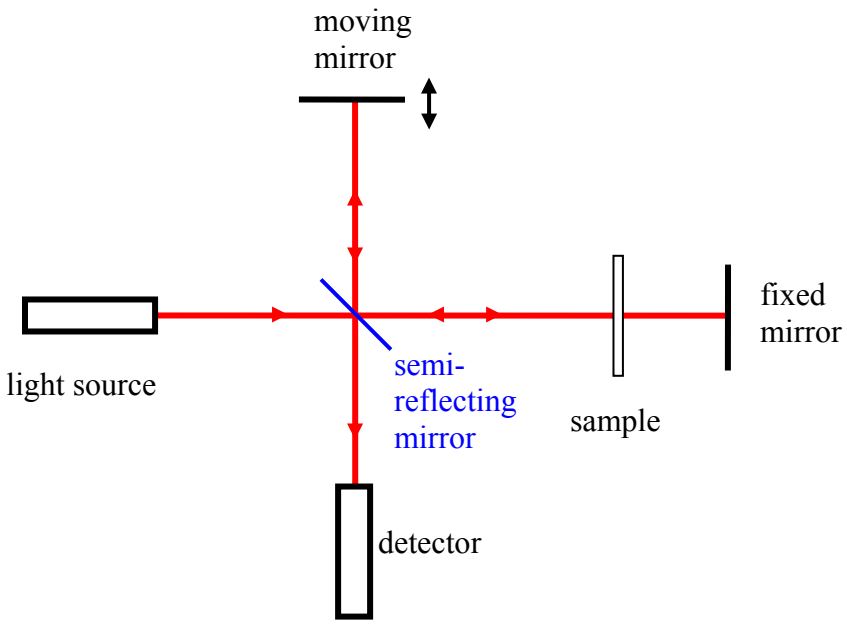


Figure 1: The Michelson interferometer setup used to measure the absorbance/ transmittance intensity I(t).

Defining the energy E, the frequency ω, the wavelength λ and the wavenumber k = 1/λ, the following relations hold for electromagnetic radiation (light):

$$E = \hbar\omega = h\frac{c}{\lambda} = hck\,. \qquad (1)$$

The FTIR method relies on performing the following Fourier transform of I(t) in order to obtain I(k):

$$I(\omega) = \int_{-\infty}^{\infty} dt\, I(t) \exp(i\omega t) \qquad (2)$$

$$I(k) = 2\pi c I(\omega) = 2\pi c \int_{-\infty}^{\infty} dt\, I(t) \exp(i\omega t).$$

Here c is the speed of light and h is Planck's constant ($\hbar = h/2\pi$). Modern FTIR instruments are effective at measuring a wide class of molecular substances in short amounts of time.

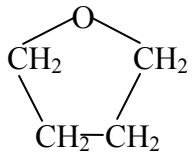
FTIR bands for the stretching modes of some common bonds are shown here:

- 3700 - 2500 cm^{-1} for H-(C, N, O or S) stretching.
- 2300 - 2000 cm^{-1} for C≡(C or N) stretching.
- 1900 - 1500 cm^{-1} for C=(C, N or O) stretching.
- 1300 - 800 cm^{-1} for C-(C, N or O) stretching.

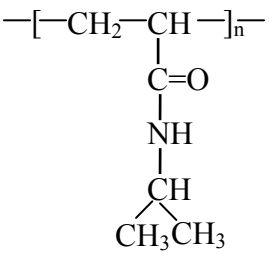
Since most organic molecules have single bonds, the region below 1500 cm^{-1} can become quite complex. The FTIR technique can distinguish between symmetric and antisymmetric bond stretching.

FTIR FROM PNIPAM IN D_2O/THF MIXTURES

Poly(N-isopropylacrylamide) also referred to as PNIPAM is a water-soluble polymer. It is rich in chemical bonds and has been the subject of FTIR studies (Maeda et al, 2000) in D_2O, in THF and in D_2O/THF solvent mixtures.



Tetrahydrofuran (THF)



Poly(N-isopropylacrylamide)
PNIPAM

Figure 2: Chemical formulas for THF solvent and PNIPAM polymer.

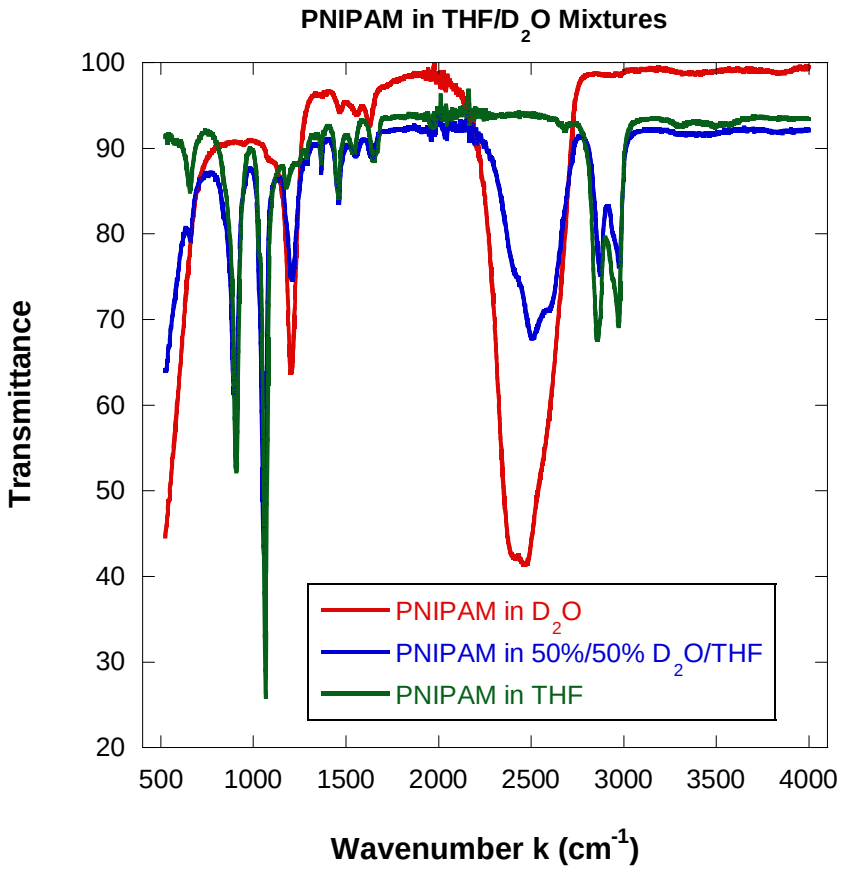


Figure 3: FTIR spectra from PNIPAM in D_2O/THF mixtures.

These spectra are complex and comprise many modes that have been discussed in the literature (Maeda et al, 2000). A table summarizes a few of these modes.

Table 1: Some FTIR lines for neat PNIPAM and PNIPAM in H_2O or THF solution

Neat PNIPAM	PNIPAM in H_2O solution	PNIPAM in THF solution	Mode assignment
2973	2982		antisymmetric C-H stretching of $-C(CH_3)_2$
2935	2940		antisymmetric C-H stretching of $-CH_2-$
2876	1880		symmetric C-H stretching of $-C(CH_3)_2$
1647		1650	amide
1460	1463	1459	symmetric deformation of $-C(CH_3)_2$
1388	1390	1386	antisymmetric deformation of $-C(CH_3)_2$
1131	1133	1130	skeletal vibration of $-C(CH_3)_2$

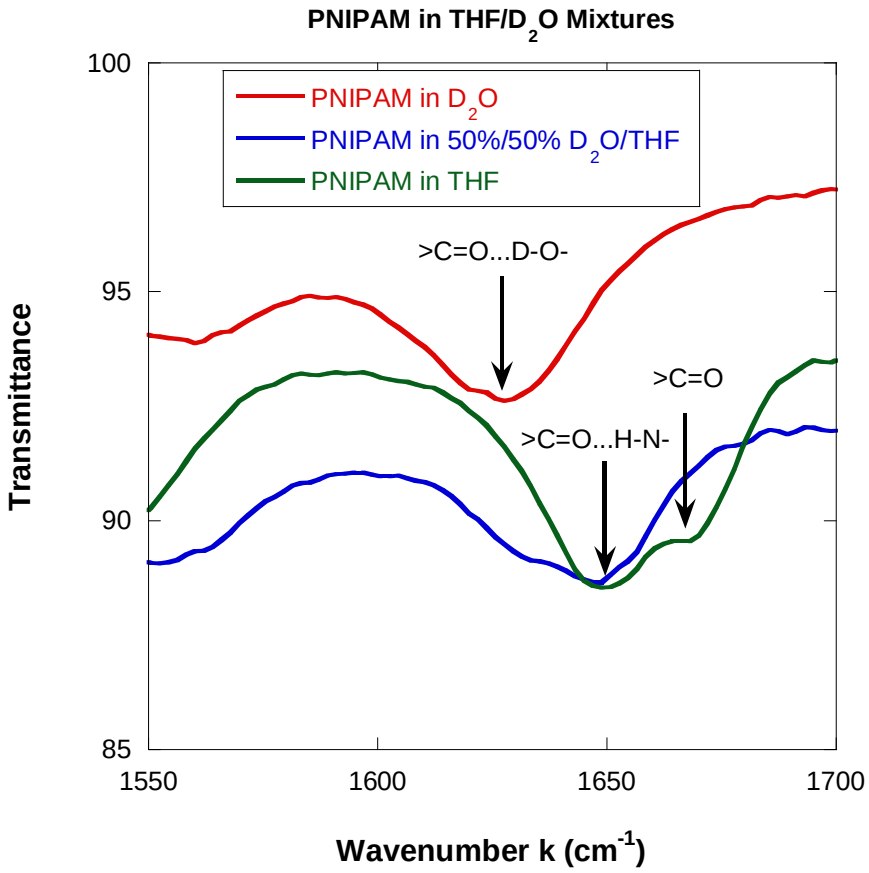


Figure 4: Limited range spectra showing a shift due to hydrogen (or deuterium) bonding.

REFERENCES

Y. Maeda, T. Higuchi, and I. Ikeda, "Change in Hydration State during the Coil-Globule Transition of Aqueous Solutions of Poly(*N*-isopropylacrylamide) as Evidenced by FTIR Spectroscopy" Langmuir 16, 7503-7509 (2000).

P.R. Griffiths, "Fourier Transform Infrared Spectroscopy", Wiley-Interscience (2007)

QUESTIONS

1. What causes infrared absorption?
2. Name a few applications of IR spectroscopy.
3. What is the basic idea behind FTIR?
4. FTIR spectra are expressed in terms of what variable (k)? What are the units of k?
5. FTIR spectra contain the "signature" of what type of molecular bonds?

ANSWERS

1. Infrared absorption is caused by molecular bond stretching.
2. IR spectroscopy is used as routine characterization method in quality control, forensic science, analytical chemistry, etc.
3. FTIR uses an interferometer to measure small time shifts characterizing bond stretching spectra.
4. FTIR spectra are expressed in terms of the wavenumber $k = 1/\lambda$ where λ is the wavelength. Note that k is expressed in cm^{-1}.
5. FTIR spectra contain the signature of covalent bonds as well weaker bonds such as hydrogen bonds.

OPTICAL MICROSCOPY

Optical microscopy was historically the first technique used to observe what could not be seen with a naked eye. Development of the magnifying glass and then of the optical microscope occurred some four hundred years ago. The first generation of (simple) optical microscopes used one focusing lens. Microscopes have evolved to using multiple (compound) lenses in order to enhance magnification.

OPTICAL MICROSCOPE BASICS

The basic principle of a compound two-lens microscope is shown here.

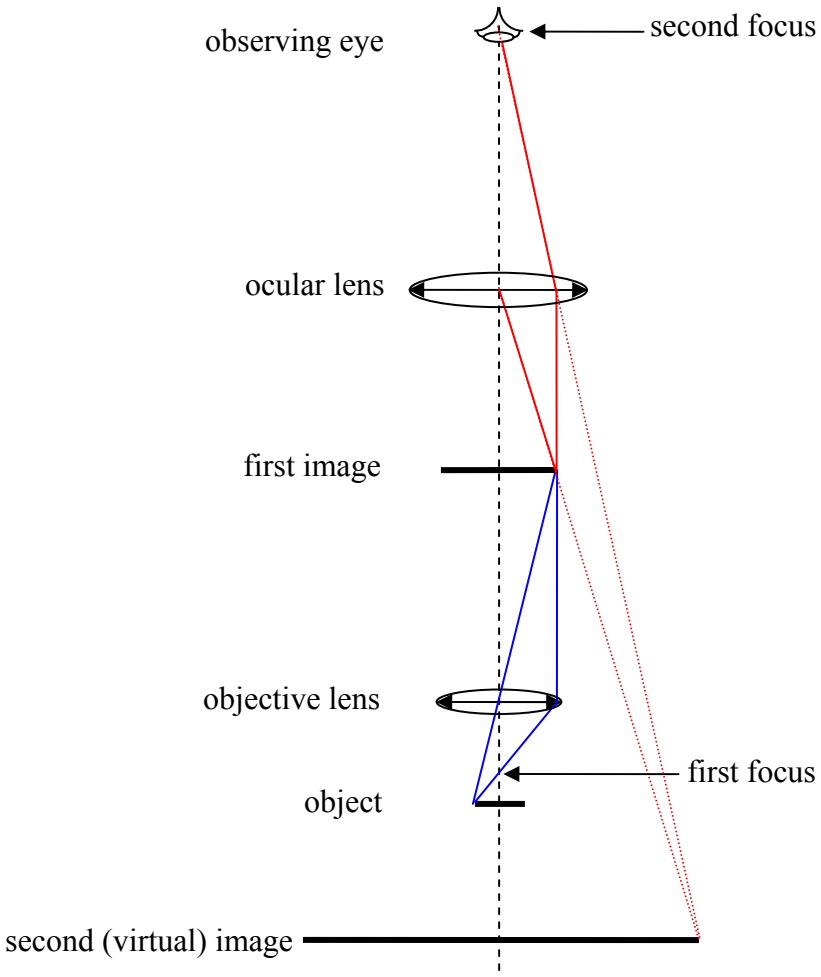


Figure 1: Schematics of a compound microscope with two lenses.

The optical microscope contains coarse and fine adjustment knobs used to bring the image of an object into focus. One of many objective lens-ocular lens pieces can usually

be used for a specific magnification. Magnification factors have improved over the years to a range between 4 and 1000. Actually, the best present-day optical microscopes can magnify up to 2000 times. The optical microscope's best resolution is around 0.2 μm. The use of CCD technology has allowed the digitization and enhanced resolution of microscopy images. Optical microscopy has become a valuable observation tool in most fields of human interest.

Many improvements have been introduced in the field of optical microscopy. For example, confocal microscopy is an optical imaging technique used to increase the micrograph contrast.

CONFOCAL MICROSCOPY FROM A SEMICRYSTALLINE POLYMER

Confocal microscopy uses point illumination and a spatial pinhole to eliminate out-of-focus light in samples that are thicker than the focal plane (Pawley, 2006). Only the light within the focal plane can be detected in order to improve the image quality. As only one point is illuminated at a time in confocal laser scanning microscopy, 2D imaging requires scanning over a regular grid in the specimen. Images are acquired point-by-point and reconstructed by software, allowing 3D reconstruction of complex morphologies.

The confocal microscopy image of a semicrystalline polymer is included (Ho et al, 2006). Poly(ethylene oxide) forms crystalline lamellae when dissolved in ethanol. The lamellae form a sponge-like morphology whereby ethanol gets trapped in the pockets that form.

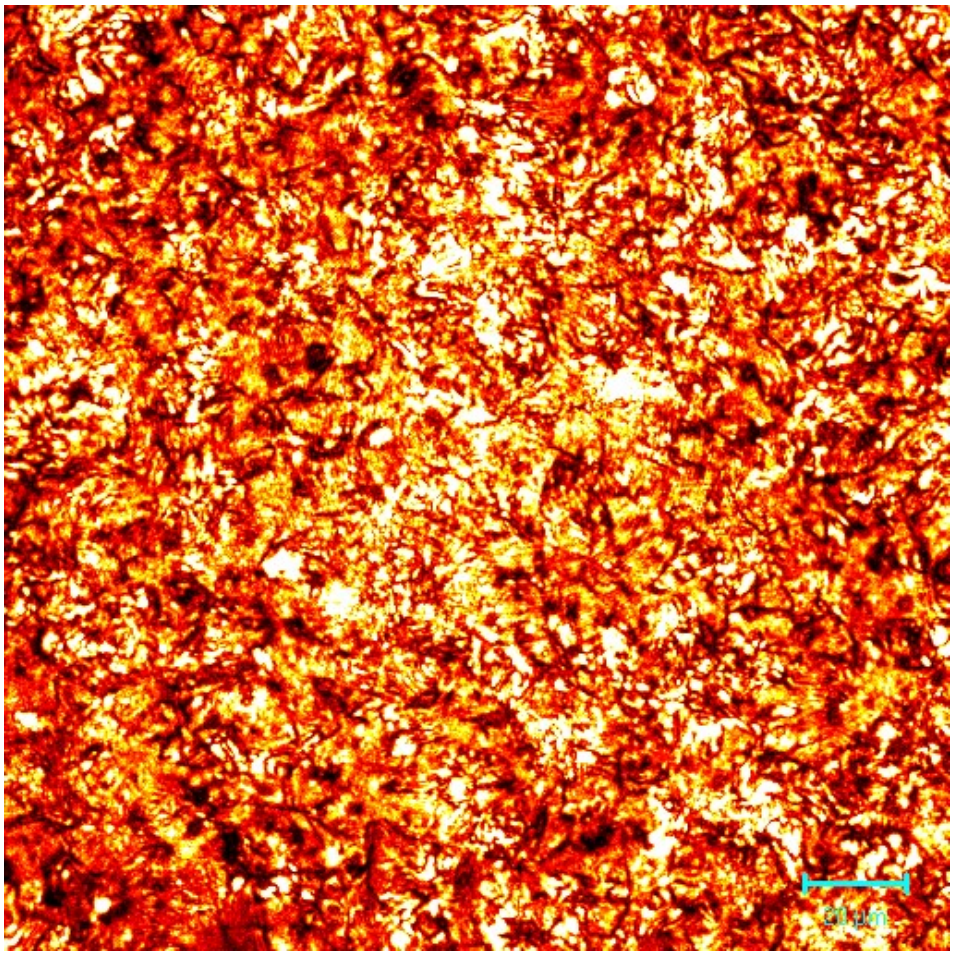


Figure 2: Confocal optical micrograph from a 4 % hPEO/h-ethanol sample. This picture represents data taken 28 μm underneath the sample surface. The scale bar represents a 20 μm length scale.

REFERENCES

J.B. Pawley, Editor. "Handbook of Biological Confocal Microscopy", 3rd ed., Berlin, Springer (2006).

D. Ho, B. Hammouda, S. Kline, and WR Chen, "Unusual Phase Behavior in Mixtures of Poly(ethylene oxide) and Ethyl Alcohol", J. Polymer Science, Polym. Phys. Ed. 44, 557-564 (2006).

QUESTIONS

1. What type of light does optical microscopy use?
2. What is the basic component that makes a microscope work?
3. What type of magnification can be obtained using typical optical microscopes?

4. What is the best resolution achieved by optical microscopes?
5. What is the limiting factor for the resolution of optical microscopes?

ANSWERS

1. Optical microscopy uses white (visible) light.
2. The focusing lens makes the optical microscope work.
3. Typical optical microscopes can achieve magnifications of order 1,000.
4. The best resolution achieved by optical microscopes is 0.2 μm.
5. The limiting factor for the resolution of optical microscopes is the wavelength of light (fraction of a micron).

ELECTRON MICROSCOPY

Electron microscopes have been around for over seventy years. The basic principle of electron microscopy is similar to that of optical microscopy; it uses a source, a set of lenses and an adjustment system to enlarge the image taken from a specimen. Electron microscopy uses electrostatic lenses to focus the electron beam and can achieve magnifications a thousand times greater than optical microscopy.

BASICS OF ELECTRON MICROSOPY

A beam of electrons is transmitted through an ultra thin specimen then focused and magnified to form an image which is displayed on an imaging screen. Unlike optical microscopes, electron microscopes produce only black and white images. The magnification of electron microscopes can be as high as 2 million times. Most ranges in the nanometer scale can be observed (Egerton, 2005).

Electrons are emitted by a cathode (usually a tungsten filament) by applying high voltage between two electrodes. The electron beam is focused and made to go through electrostatic lenses to produce an image which is recorded by hitting a fluorescent screen, a photographic plate, or a light sensitive sensor such as a CCD; this is displayed in real time on a monitor.

There are two main types of electron microscopy: Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). TEM works in transmission geometry and requires (microtomed) thin samples whereas SEM measures low energy secondary electrons emitted from the specimen surface due to excitations in the specimen itself produced by the primary electron beam. SEM is not a surface reflection method but can image surfaces with excellent resolution. SEM can achieve magnifications an order of magnitude higher than TEM. The TEM resolution is limited by spherical aberration, which can be corrected by software in state-of-the-art machines.

There are a number of drawbacks to the TEM technique. The extensive sample preparation effort required makes this technique highly time consuming. Moreover, the fact that electrons are highly attenuated when going through any window material limits the number of possible sample environments. For example, elaborate heating or pressure cells cannot be used in-situ. Finally, the drastic effect from the bombarding electron beam damages samples particularly in the case of delicate biological materials.

In order to enhance the resolution of the technique, some special sample preparation methods are used. Staining uses heavy metals (such as lead, tungsten or ruthenium) to enhance the contrast of one phase in the sample.

Cryo-TEM uses a copper grid to support a thin carbon film that produces weak contrast without staining. The thin (100 μm thickness) sample is blotted first to remove excess

solvent, then quenched in liquid ethane (-175 ^{o}C). This vitrifies the sample to suppress water crystallization. The sample is then transferred to the Cryo-TEM holder which is maintained at liquid nitrogen temperature.

Electron microscopy has affected most fields of research and development.

TEM FROM A SEMICRYSTALLINE POLYMER

Poly(ethylene oxide) crystallizes in ethanol solution even at low concentrations. The sample was stained in order to enhance the contrast between the crystalline and amorphous structures. The formed morphology is sponge-like with crystalline lamellae forming partitions between pockets of solvent. A TEM image from a 4 % PEO/ethanol is shown.

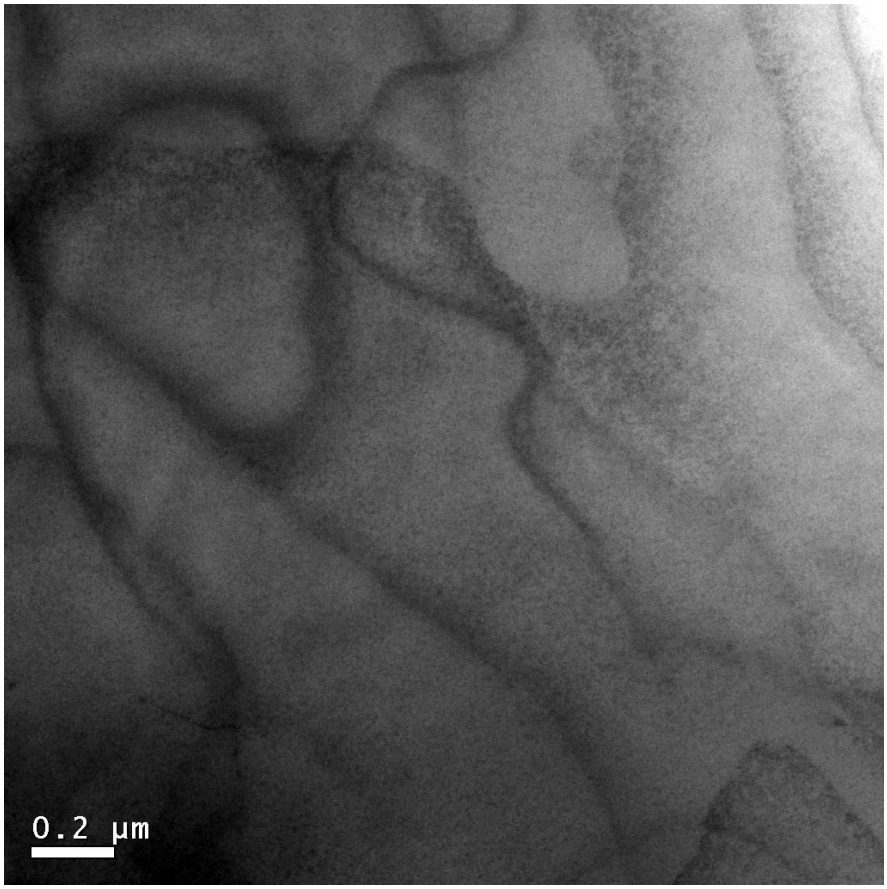


Figure 1: Transmission Electron Micrograph (TEM) of a 4 % PEO/ethanol semicrystalline sample. The sample was stained. The sponge-like crystalline structure traps pockets of solvent. The crystalline lamellae form the partitions.

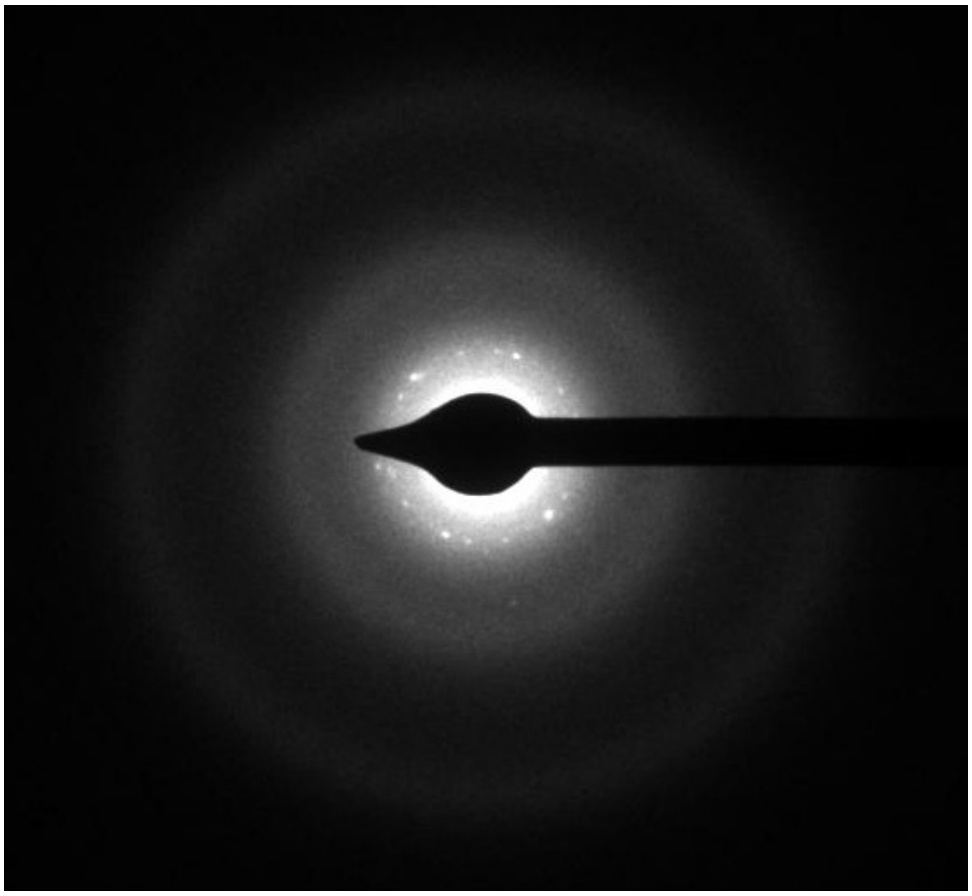


Figure 2: Electron diffraction micrograph from the same 4 % PEO/ethanol semicrystalline sample. The diffraction spectrum shows strong low-Q scattering and a series of peaks forming a ring at Q = 0.05 $Å^{-1}$.

CRYO-TEM FROM VESICLES

Sample preparation for Cryo-TEM involves laying the TEM grid on a glass slide. The grid is then vitrified immediately in liquid ethane (185 K = -88 ^{o}C) before the hydrogel dries out. A Cryo-TEM image from vesicles produced by bacterial surfactants (marinobactins) is shown. This image shows vesicles with an average diameter around 100 nm (Owen et al, 2007).

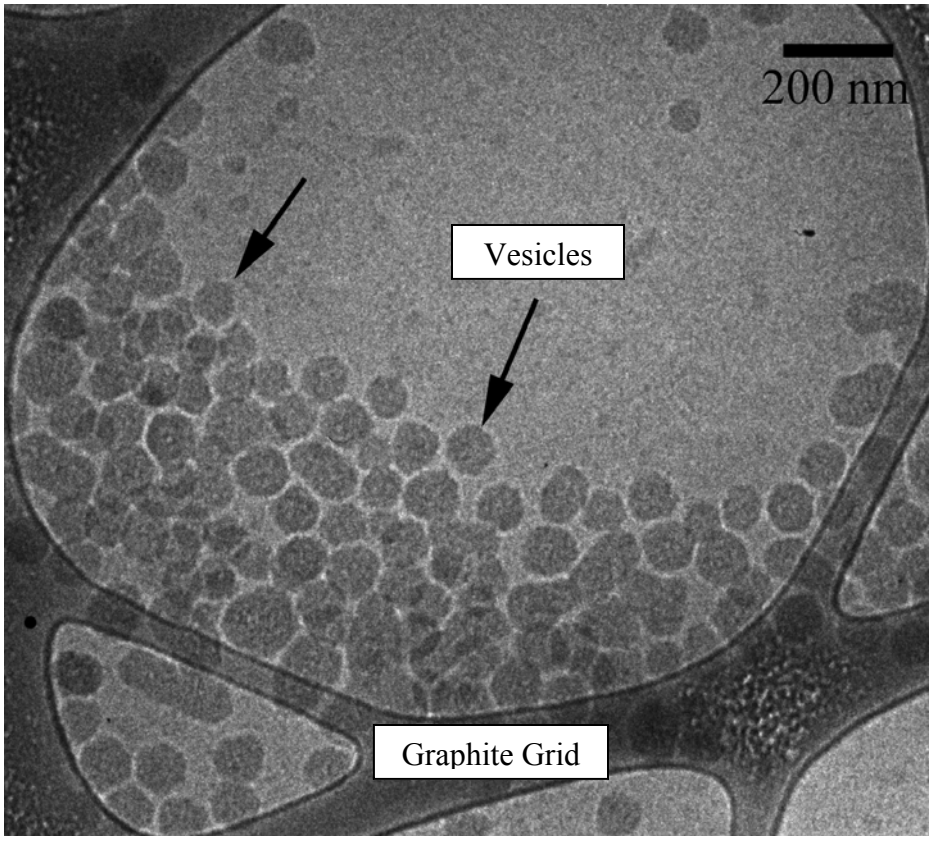


Figure 3: Cryo-TEM micrograph of a marinobactin-produced vesicle morphology. Arrows indicate representative particles. The graphite grid is seen as dark background.

REFERENCES

R.F. Egerton, "Physical Principles of Electron Microscopy: An Introduction to TEM, SEM, and AEM", Springer (2005).

T. Owen, R. Pynn, B, Hammouda and A. Butler, "Metal-Dependent Self-Assembly of a Microbial Surfactant", Langmuir 23, 9393 - 9400 (2007).

QUESTIONS

1. What type of lens does an electron microscope use?
2. What magnification can electron microscopes achieve?
3. What are the two types of electron microscopes?
4. What is the main disadvantage of electron microscopy?
5. What is the main advantage of microscopy (as compared to scattering methods).

ANSWERS

1. Electron microscopes use electromagnetic lenses.
2. Electron microscopes can achieve magnifications as high as 2 million.
3. The two types of electron microscopes are: Scanning Electron Microscope (SEM) for surfaces and (2) Transmission Electron Microscope (TEM).
4. Electron microscopy uses only very thin samples (films).
5. Microscopy probes the direct space whereas scattering methods probe the reciprocal space.

CALORIMETRY

Calorimetry is the analytical method of measuring the heat emitted and/or absorbed during thermodynamic transitions or chemical reactions. Heat, internal energy and work of a closed system are related by the following thermodynamic relations:

$$Q = U - W \qquad (1)$$
$$H = U + PV .$$

Here Q is the heat, U is the internal energy, W is the work performed on the system, H is the enthalpy, P is the pressure, and V is system volume. The most commonly used form of calorimetry is the differential scanning calorimetry.

DIFFERENTIAL SCANNING CALORIMETRY

Differential Scanning Calorimetry (DSC) measures the amount of heat absorbed or emitted by a system as well as by a "reference" sample undergoing heating and/or cooling cycles. The difference in the amount of heat required to increase the temperature of both the measured and reference samples is measured as a function of temperature. Both samples are maintained at nearly the same temperature throughout the measurement (Dean, 1995).

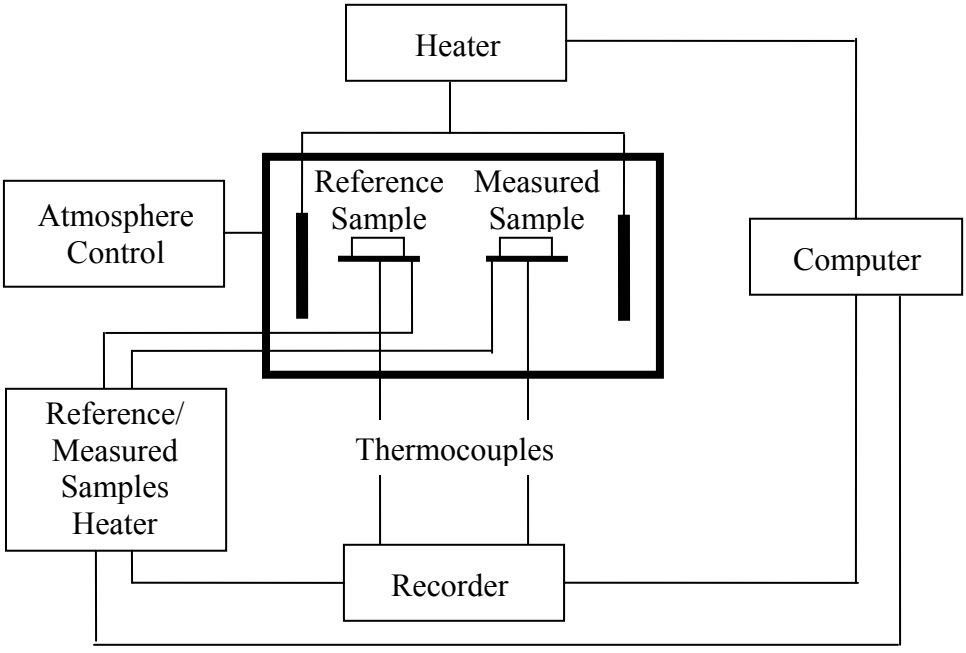


Figure 1: Schematics of the components of a DSC instrument.

The measured heat flow is either positive or negative depending on whether the measured sample gives off or receives heat during the various transitions. The process of crystallization, for example, is exothermic whereas melting is endothermic.

Many thermodynamic processes can be analyzed using DSC. Among these are the glass transition, crystallization, phase separation and melting. DSC is routinely used for research and quality control in many areas including drug analysis, food science, polymer characterization, materials science, liquid crystals, etc.

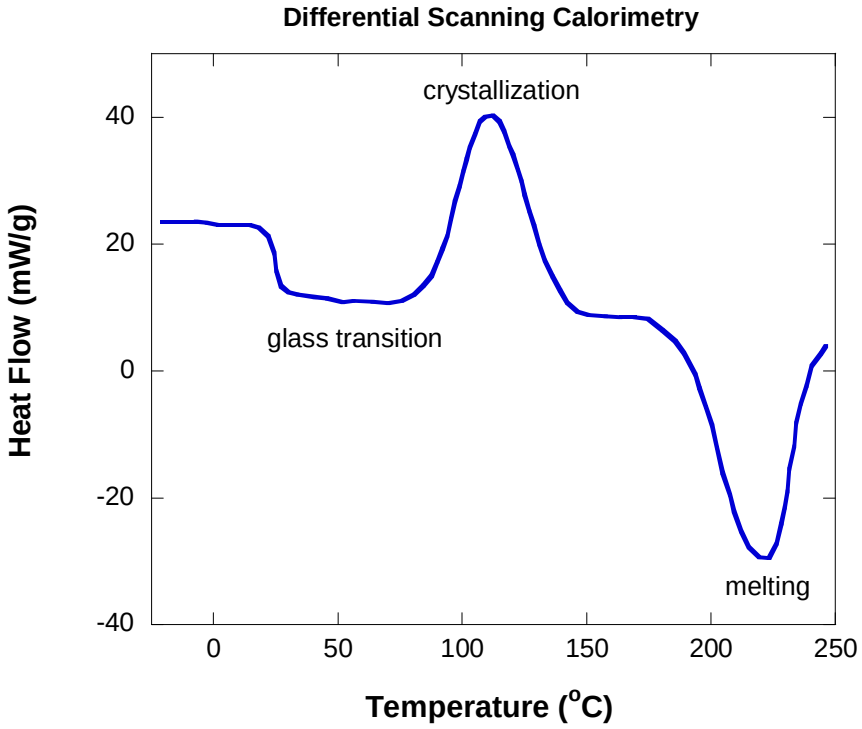


Figure 2: Generic DSC trace for a fictitious polymer showing the main phase transitions characterizing polymers.

Enthalpies can be calculated by integrating the area underneath a peak in the DSC trace.

DSC FROM A SEMICRYSTALLINE POLYMER SOLUTION

The DSC curve for a semicrystalline solution of poly(ethylene oxide) in d-ethanol (Ho et al, 2006). Deuterated ethanol was used in order to be consistent with SANS data taken on the same system. The DSC curve shows a crystal melting process upon heating (around 60 ^{o}C) and a crystallization process upon cooling (around 45 ^{o}C) of the PEO.

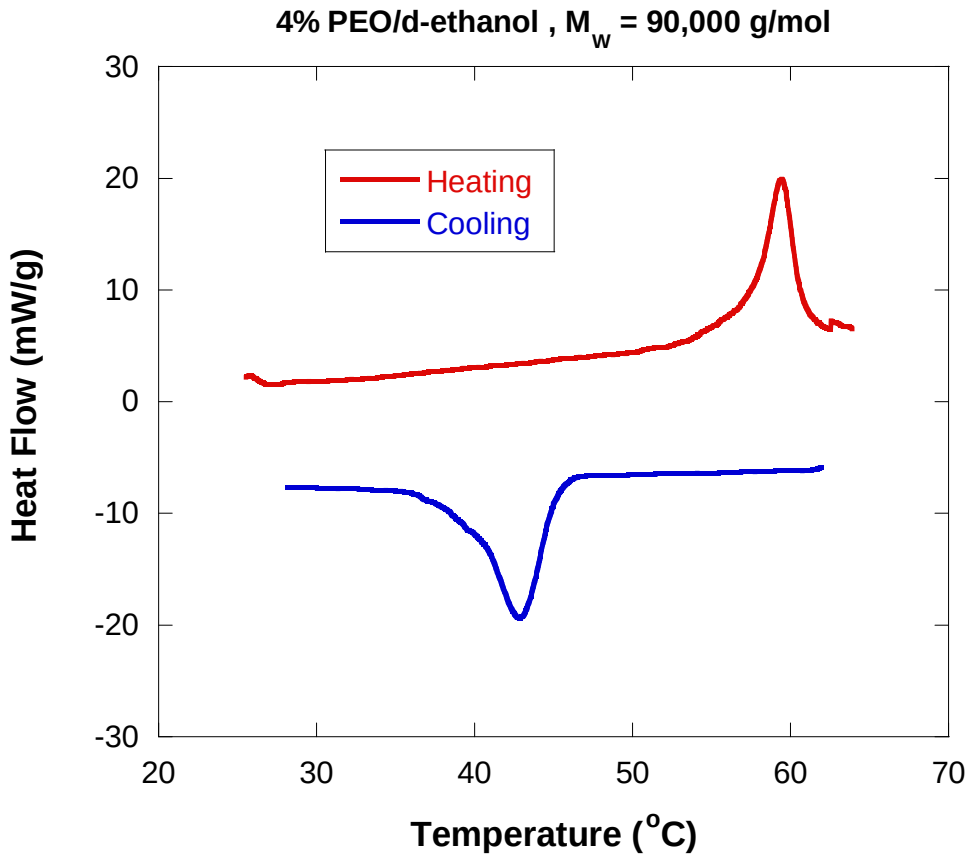


Figure 3: Differential Scanning Calorimetry (DSC) measurements from 4 % PEO/d-ethanol showing the crystalline nature. The heating and cooling curves show the effect of melting and crystallization.

REFERENCES

J.A. Dean, "The Analytical Chemistry Handbook". New York, McGraw Hill (1995).

D. Ho, B. Hammouda, S. Kline, and WR Chen, "Unusual Phase Behavior in Mixtures of Poly(ethylene oxide) and Ethyl Alcohol", J. Polymer Science, Polym. Phys. Ed. 44, 557-564 (2006).

QUESTIONS

1. What does calorimetry measure?
2. What type of transitions can calorimetry detect?
3. What is the basic principle of Differential Scanning Calorimetry?
4. Is DSC a thermodynamic or a spectroscopic probe?
5. What are thermocouples used for?

ANSWERS

1. Calorimetry measures the amount of heat absorbed or produced during phase transitions.
2. Calorimetry can detect the glass transition, crystallization and melting among others.
3. Differential Scanning Calorimetry (DSC) measures the amount of heat absorbed or produced by a system as well as by a "reference" sample undergoing heating and/or cooling cycles.
4. DSC is a thermodynamic probe. It probes a macroscopic property of the sample.
5. Thermocouples are used to measure in-situ temperature.

CHROMATOGRAPHY

BASICS OF CHROMATOGRAPHY

Chromatography is a separation method for measuring molecular weight distributions. It consists in passing a mixture containing an unknown molecular weight distribution through a vertical column. The rate of diffusion through the column depends on the molecular weight with small molecules diffusing slower than large ones. Chromatography is also used for fractionation into many narrow molecular weight fractions. Interaction between the diffusing sample and the absorbing substance forming the column (such as silica) is the driving force behind the separation process. As the sample components elute from the column, they are quantified by a detection system and/or collected in separate containers. The resulting chromatography data represents the relative fraction (or concentration) *vs* retention time (or molecular weight).

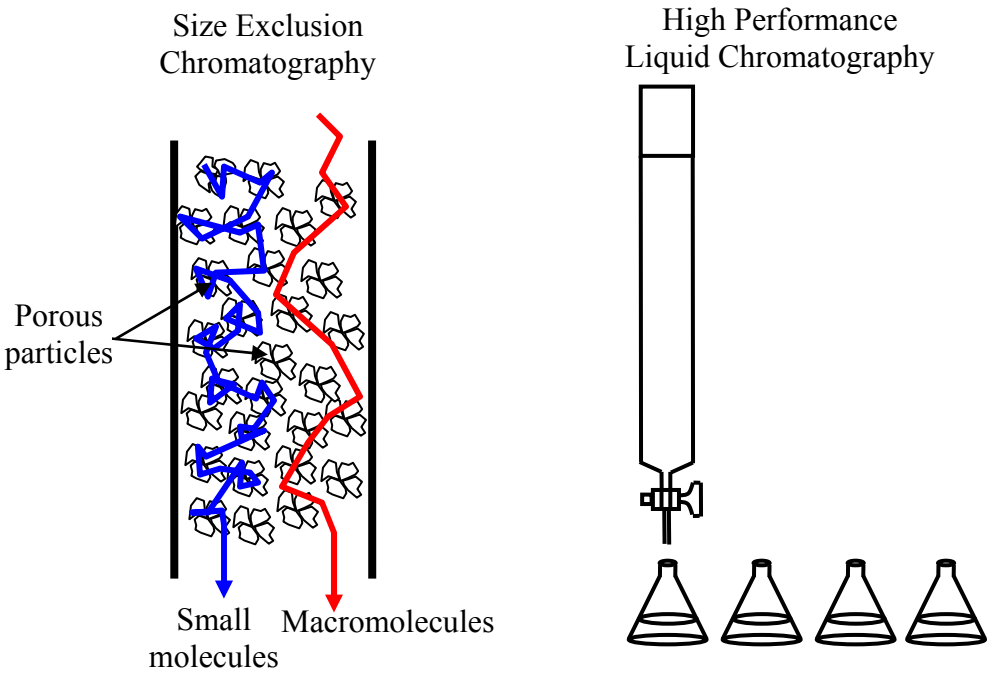


Figure 1: Schematics of chromatography.

THE VARIOUS TYPES OF CHROMATOGRAPHY

Size Exclusion Chromatography (SEC) also known as Gel Permeation Chromatography (GPC) separates molecules according to their size. Smaller molecules can enter the pores of the column-filling gel and, therefore, take longer time to elute, whereas larger

molecules do not enter the pores and elute faster. Flash column chromatography applies external pressure to reduce the diffusion time down the column.

In High Performance Liquid Chromatography (HPLC), the column is filled with small packing particles. HPLC uses high pressure to force the solvent through the closed column. This technique is used for separation by composition and is effective for fractionation of multicomponent mixtures.

Thin Layer Chromatography (TLC) uses a thin layer of the adsorbent gel (like silica) on a flat substrate. In Gas-Liquid Chromatography (GLC), the mobile phase is a gas (such as helium) and the stationary phase is a liquid (such as liquid silicone).

Ion Exchange Chromatography (IEC) uses a charged stationary phase (such as ion exchange resin with charged groups) to separate charged macromolecules such as amino acids and proteins. This technique is commonly used to purify proteins.

SEC FROM A POLYMER

Size Exclusion Chromatography (SEC) was carried out on a Varian liquid chromatograph equipped with a refractive detector. The GPLC column was used at 30 ^{o}C with THF as the eluent. The column was first calibrated using monodisperse poly(ethylene oxide) standards. The molecular weights and the polydispersity index of the measured poly(ethylene oxide) polymer were determined. The polydispersity index is given by the ratio $PDI = M_w / M_n$ where M_n is the number average and M_w is the weight average molecular weights defined as:

$$M_n = \frac{\sum_i N_i M_i}{\sum_i N_i}, \; M_w = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i}. \qquad (1)$$

The SEC trace for the measured PEO is included here.

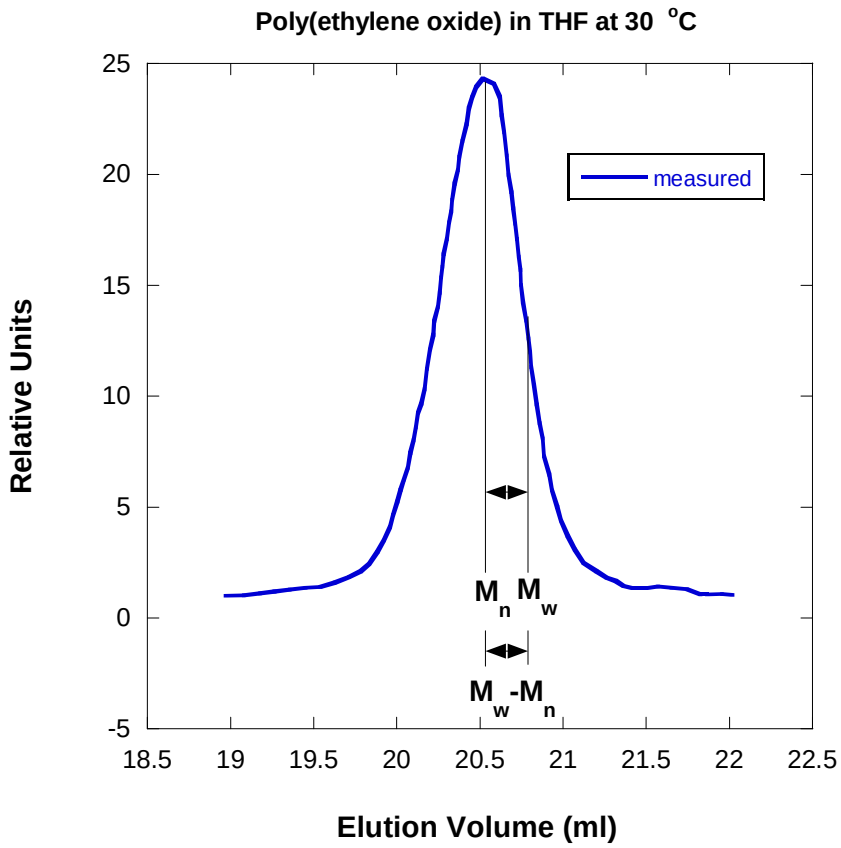


Figure 1: SEC from poly(ethylene oxide). M_w = 100,000 g/mol, M_n = 96,000 g/mol, polydispersity index =1.04.

REFERENCES

E Katz, "High Performance Liquid Chromatography: Principles and Methods in Biotechnology", Wiley (1996)

S Mori, "Size Exclusion Chromatography", Springer (1999)

QUESTIONS

1. What is the basic idea behind chromatography?
2. What is another name for Gel Permeation Chromatography?
3. What chromatography method is used to determine polymer molecular weights?
4. What is the polydispersity index?
5. What is fractionation?

ANSWERS

1. Chromatography consists mainly in letting a substance diffuse down a column. The molecular size and interactions with the column filler dictate their diffusion time.
2. Gel Permeation Chromatography (GPC) is also called Size Exclusion Chromatography (SEC).
3. The GPC (or SEC) method is used to determine polymer molecular weight.
4. The polydispersity index is given by $PI = M_w / M_n$ where M_w is the weight average and M_n is the number average molecular weights.
5. The process of fractionation consists in separating the components of a molecular mixture based on their diffusion time down a chromatographic column.

DENSITY MEASUREMENTS

The density of a material is the ratio of its mass to its volume. Density measurements involve either precise measurements of the mass and volume or other indirect methods for measuring physical quantities that depend on density. Density measurement methods are also referred to as densitometry.

VARIOUS DENSITY MEASUREMENT METHODS

The most common instrument for measuring densities is the hydrometer. This instrument measures the relative density of liquids (with respect to that of water). A hydrometer consists of a cylindrical stem made out of glass and a bulb weighed by a heavy metal (mercury or lead) to keep it floating upright. The measured liquid is poured into a jar, and the hydrometer is lowered into the liquid until it floats. The stem of the hydrometer has a graduation (ruler) noting its depth level. The principle of the hydrometer is based on the Archimedes principle which states that the buoyancy force pushing the hydrometer upward is proportional to the weight of the displaced liquid. This yields a simple (and crude) way of measuring relative densities. In light liquids, the hydrometer sinks deeper than in heavy liquids.

Another simple (and old) instrument used to measure density is the pychnometer. It is a glass flask capped with a tight fitting that has a capillary tube through it to allow air bubbles to escape. The pychnometer is weighed empty first, then full of water (known density), and then full of the liquid whose density is being measured. Since the same volume is filled each time, the density (relative to that of water) is measured. Precision depends on accuracy of the weighing balance.

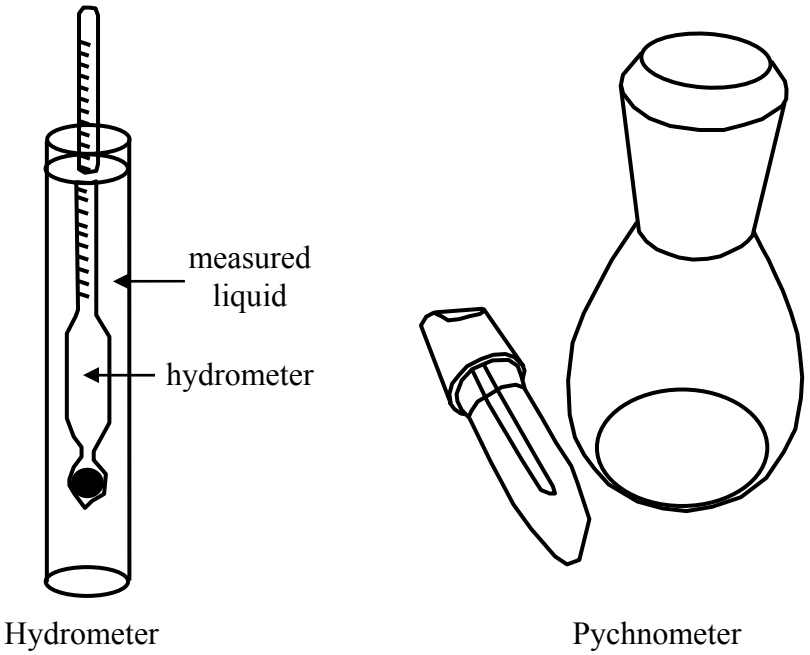


Figure 1: Schematics of the hydrometer and the pychnometer densitometers.

Another instrument used to measure the density of liquids (and gases) is the vibrating U-tube described next.

THE VIBRATING U-TUBE METHOD

The vibrating U-tube density measurement method (Stabinger, 1994) is based upon measuring the frequency of oscillation of a U-shaped tube filled with the measured liquid (or gas). The vibrating tube is likened to a vibrating mass spring. The frequency of oscillation f depends on the vibrating mass m and spring constant k as $f = (1/2\pi)\sqrt{k/m}$. Since the same volume is involved, a precise measurement of the mass yields the density with high precision. This method can be used even for flowing liquids.

The vibrating U-tube is driven by an electro-mechanical system that uses a magnet and coil assembly. The coil creates an oscillating magnetic field that acts (pushes) on the magnet and therefore on the tube periodically. A feedback amplifier is used to maintain the oscillation at the resonant frequency. The mechanical oscillation of the tube is transformed into an alternating voltage of the same frequency and then to a calibrated digital signal of the density. The calibration is performed using substances of precisely known densities.

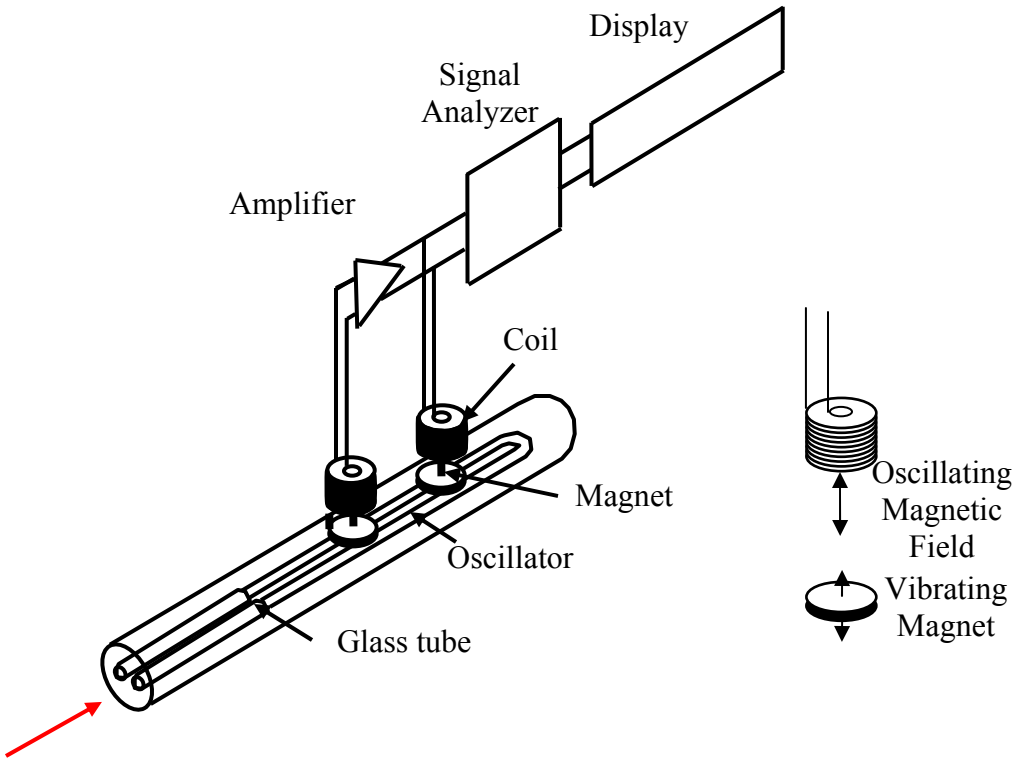


Figure 2: Schematic representation of the vibrating U-tube instrument.

DENSITY MEASUREMENTS OF A SEMICRYSTALLINE POLYMER

Precise density measurements can monitor structural and thermodynamic changes in the sample. For example, density measurements can determine the crystal melting temperature (while heating) and the crystallization temperature (while cooling) in crystalline materials. Poly(ethylene oxide) forms a crystalline structure in ethanol. The density of 4 % PEO in d-ethanol is plotted for increasing and decreasing temperature. The onset of crystal melting is observed at 35 ^{o}C and melting is complete for 40 ^{o}C. Crystallization occurs for temperatures below 28 ^{o}C.

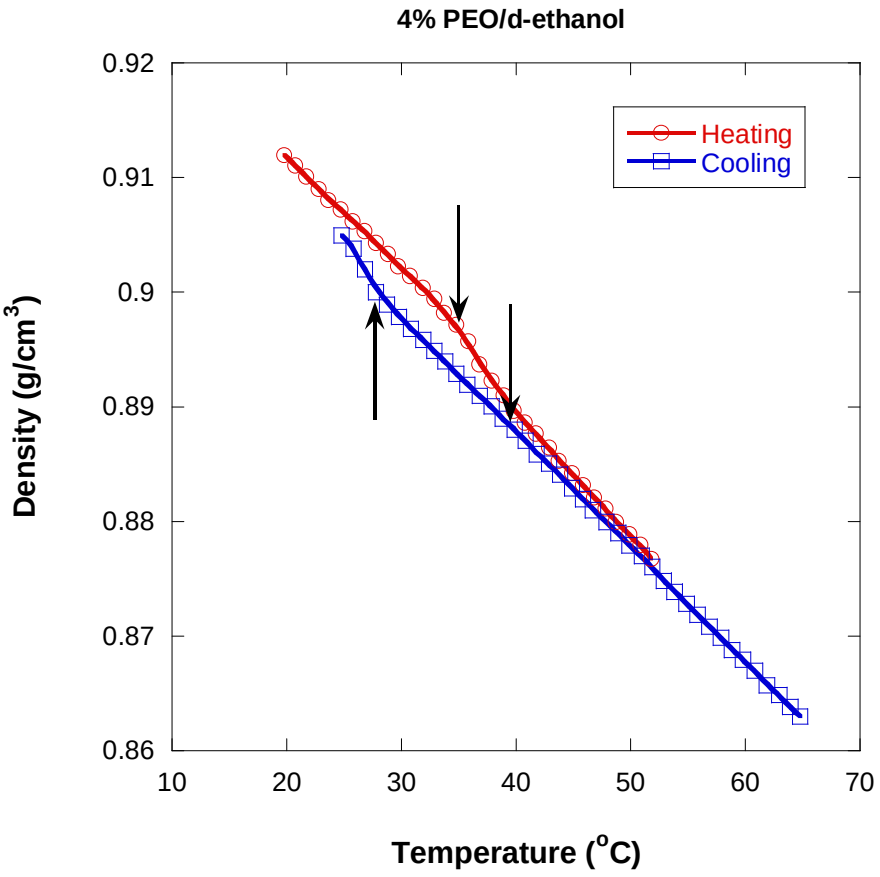


Figure 3: Density measurements for 4 % PEO/d-ethanol. The heating and cooling cycles are shown. Arrows show breaks in the data trend corresponding to melting and crystallization transitions.

REFERENCES

H. Stabinger, "Density Measurement using Modern Oscillating Transducers", South Yorkshire Trading Standards Unit, Sheffield (1994)

S.V. Gupta, “Practical Density Measurement and Hydrometry”, Institute of Physics Pub, (2002)

QUESTIONS

1. Define the density of a material.
2. How is the density measured?
3. What is the basic principle behind the vibrating U-tube method?
4. How is the vibration applied to the vibrating U-tube?
5. What is the resonant frequency?

ANSWERS

1. The density of a material is the ratio of its mass to its volume.
2. The density is measured by measuring the mass for fixed volume.
3. The vibrating U-tube method is based on the principle of the vibration of a spring (the vibration frequency depends on its mass).
4. The vibration to the U-tube is applied using an electro-mechanical method. An alternating current of variable frequency flows inside a coil to produce an oscillating magnetic field which acts (pushed and pulls) on a magnet.
5. The resonant frequency is the largest frequency at which the U-tube responds to the applied electro-mechanical vibration.

MORE CHARACTERIZATION METHODS

There are many more characterization methods that were not covered in this tutorial. Some of these are mentioned briefly here.

RHEOLOGY

Rheology is the investigation of flow and deformation of matter under the influence of an external periodic stress or flow field. Response is either Newtonian (for small molecular fluids) or non-Newtonian (for macromolecular or self-assembled fluids). One of the goals of rheology is to establish relationships between deformation or flow and applied stress. Rheometers are operated either in a constant stress or constant shear mode.

There are many types of rheometers including the Couette type (for which a "bob" rotates inside a fixed cylindrical cup full of sample), the cone-and-plate type (where a solid cone rotates on top of a flat horizontal surface), the Poiseuille type (where the fluid is kept flowing continuously between two fixed confining surfaces), the plate-and-plate type (where shear is applied to a reciprocating plate while the second plate is kept fixed), etc.

Some fluids are characterized by shear thinning for which the viscosity decreases with shear rate or by shear thickening for which the viscosity increases with shear rate.

NUCLEAR MAGNETIC RESONANCE

Nuclear Magnetic Resonance (NMR) measures the spin response of nuclear moments to applied magnetic fields within molecules. This probes the crowding environment around specific bonds. For example, proton NMR is sensitive to environments around hydrogen atoms. Carbon-13 (as well other types of NMR techniques) is also used.

The NMR technique consists in aligning the magnetic moment of nuclei using both a constant magnetic field and an (orthogonal) alternating magnetic field. The response of the nuclear moment in the form of a "chemical shift" yields a unique signature of the neighboring bonding environment. This chemical shift is the result of the so-called Zeeman splitting.

MASS SPETROSCOPY

Mass spectroscopy is an analytical method used to identify the composition of a compound based on its mass-to-charge ratio of the charged particles. Chemical fragments of the sample are produced through bombardment from an ion source. They are then accelerated using an electric field and passed through a magnetic field that curves their

trajectory; the heavier the fragment, the larger the trajectory radius. The abundance of the various fragments is determined.

Matrix-Assisted Laser Desorption/Ionization (MALDI) is a soft ionization technique used to achieve high resolution mass spectroscopy for synthetic and biological macromolecules. The ionization is produced by a laser beam (instead of an ion source). A matrix is used to protect the macromolecules from being destroyed by the direct laser and to facilitate vaporization and ionization.

ATOMIC FORCE MICROSCOPY

Atomic Force Microscopy (AFM) is a high resolution surface scanning method. It consists of a cantilever with a sharp probe at the end used to scan the flat surface without "touching" it. Atomic (Angstrom) scale resolution can be achieved. When the probe tip is brought close to the sample surface, forces between the tip and the sample lead to a minute deflection which can be measured for example using sensitive laser reflection. Another sensitive way of measuring the deflection consists in using a cantilever fabricated from a piezoresistive element that acts as strain gauge. A feedback mechanism is used to adjust the tip-to-sample distance in order to maintain a constant force between the tip and the sample. The height of the surface inhomogeneities are mapped into an x-y-z topographical map.

MULTIPLE CHARACTERIZATION METHODS

Some analytical characterization instruments include more than one characterization method applied on a single sample. A possible multilevel chromatographic sequence is described. In this sequence, the sample is first separated (fractionated) using HPLC, then analyzed by molecular size and weight using GPC. A UV detector constitutes the first spectroscopic characterization, followed by one of more of the following methods: analysis of composition by FTIR, analysis of branching, etc by NMR, determination of absolute molecular weight by DLS and analysis of possible chemical headgroups using the high resolution mass spectroscopy (MALDI).

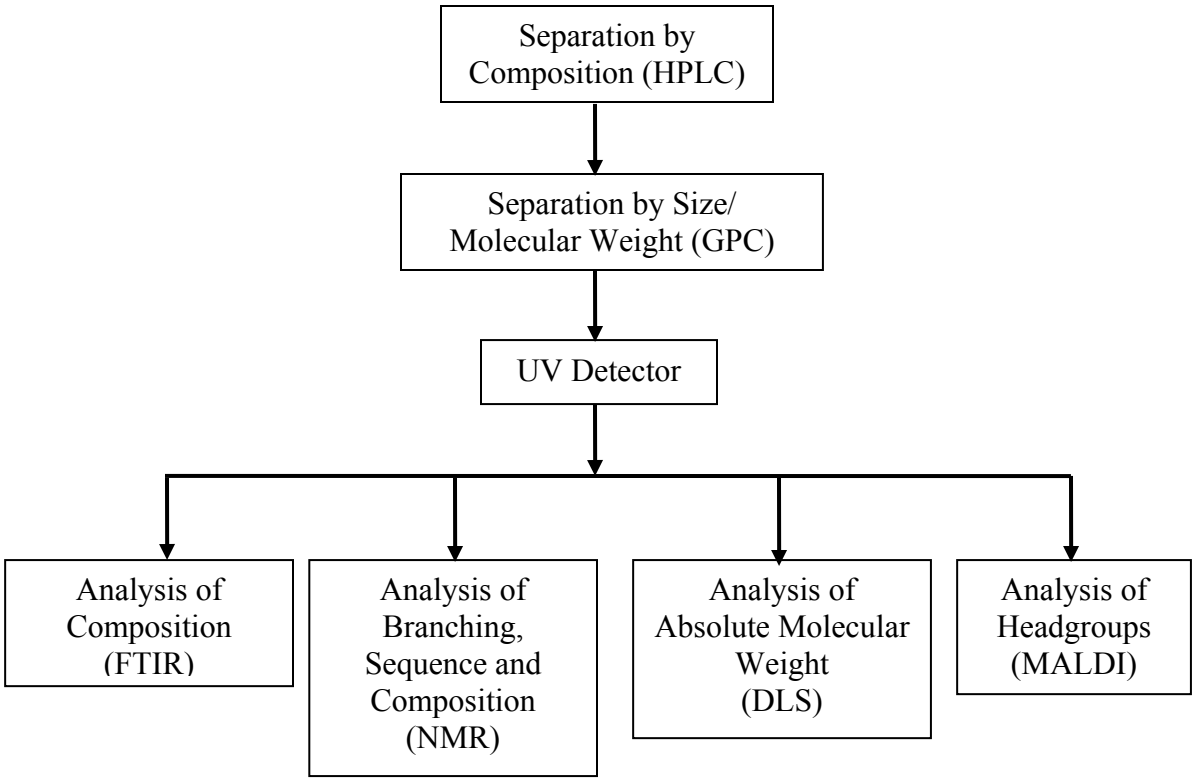


Figure 1: Multilevel chromatographic sequence.